RECONCILING PH IN RECIRCULATING AQUAPONIC SYSTEM IMPACTING NITRIFICATION AND PEPPER YIELD.

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI‘I AT MANOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN MOLECULAR BIOSCIENCES AND BIOENGINEERING

DECEMBER 2016

By Mahrukh Khawaja

Thesis Committee:

Jon Paul Bingham, Chairperson

Theodore Radovich

Bradley Fox

Keywords: recirculating aquaponics, water conservation, capsaicin, sustainable agriculture

Acknowledgements

I dedicate this thesis to my Mom who continues to support me in all my endeavors.

Many people have assisted my research and writing. It is my pleasure to acknowledge the following individuals and institutions for their help and information. First I would like to thank my committee members, Dr. Jon-Paul Bingham, Dr. Theodore Radovich, and Dr. Bradley Kai Fox for their guidance and continued support. Kai was my mentor from the beginning and with his guidance I was able to construct the foundation of my experiment. Ted was a valuable source for understanding pepper yield and quality. And J.P. was gracious enough to allow me to use his lab equipment and materials to complete the final touches of my experiment.

I thank these individuals for their encouragement and constructive comments throughout my time at the University. My original PI from the very beginning: Clyde Tamaru, because of his efforts I was able to conduct research in aquaponics. RuthEllen Klinger Bowen was another valuable resource at Hale tuahine in regards to water quality and fish behavior. I deeply thank Maile Goo for her encouragement and advice in a range of matters; I thank her for all she had done. Also Leina'ala Bright and my undergraduate volunteers Tiffany Ulep (MBBE), Koa Webster (International Business), Christian Mathias (TPSS). I'd like to thank the aquaponic farming community in Hawaii for sharing their advice and knowledge throughout the years. Hawaii Backyard and Commercial Farmers: Happyponics, Kahumana, Mari's, Gigi's Farm, Olomana Gardens and Hawaii Aquaculture and Aquaponics Association. To the organizations and the individuals named I am deeply grateful.

Finally I would like to thank my funding sources for supporting my projects. This research could not have been done without the financial assistance of Horimasa, Hatch, and SEED - GPA. I especially thank my Parents for giving me the financial opportunity to study across the world. In a first generation Asian culture where medical school and law school are the only options females have. They gave me the opportunity to pursue my own interests, for which I am forever grateful.

Abstract

The effective use of land for maximal food production is a forever-increasing worry to islands in the Pacific, which have experienced rapid population growth. To address this we examine linked fish and vegetable production using a recirculating water system. This system is designed to achieve a high degree of efficiency of water use for food production without soil. Twenty-four identical systems were used, in which each system contained a biomass of 1.5-kg tilapia species (*Oreochromis spp.*) grown in 400-L freshwater tanks associated with two ebb-and-flow 25-L bio-filters (cinder rocks). *Capsicum frutescens* (Hawaiian chili) was cultivated in these experimental aquaponic systems and analyzed for capsaicin content. The purpose of this investigation was to: 1) obtain baseline water quality criteria 2) remediate pH for ammonia bio-filtration and pepper yield in recirculating aquaponic system in order to compare buffering capacity and understand treatment effect, and 3) quantify and compare capsaicinoid concentration between treatments using Rapid-High Performance Liquid Chromatography (r-HPLC) for quality analysis. This work helps address the need for combined approaches to complex agricultural research questions and food sustainability.

List of Tables Page

List of Figures Page

List of Abbreviations and Symbols

rp-HPLC…………reverse phase high performance liquid chromatography
USDA…………United States Department of Agriculture
FAO…………Food and Agriculture Organization
ASC…………Aquaculture Stewardship Council
NOAA…………National Ocean and Atmospheric Administration
RAS…………recirculating aquaculture system
N_2O…………nitrous oxide
NUE…………nitrogen utilization efficiency
NO_3-…………nitrate
N_2…………nitrogen gas
N…………nitrogen
P…………potassium
WWOOF…………worldwide organic opportunities on farms
K_2CO_3…………potassium carbonate
$CaCO_3$…………calcium carbonate
$Ca(NO_3)_2$…………calcium nitrate
KNO_3…………potassium nitrate
CRD…………complete randomized design
FCR…………feed conversion ratio
K…………condition factor
NH_3…………ammonia
NH_4+…………ammonium
AOB…………ammonia oxidizing bacteria
NOB…………nitrite oxidizing bacteria
TAN…………total ammonia nitrogen
O_2…………oxygen
DO…………dissolved oxygen
EC…………electrical conductivity
R…………carbon chains
Ca…………calcium
PDA…………photodiode array detector
TEA…………triethylacetate
uL…………microliter
ppm…………parts per million
ANOVA…………analysis of variance
p…………probability
R^2…………coefficient of determination (correlation)
CV…………coefficient of variation
SD…………standard deviation
N…………number of variables
NC…………noridyhydrocapsaicin
C…………capsaicin
DC…………dihydrocapsaicin
SHU…………Scoville Heat Units

TRT……………………………………………………………………….treatment
ND……………………………………………………………………..not determined
ASTA…………………………………………..American Spice Trade Association
PAL………………………………………………….phenylalanine ammonia-lyase
NaCl…………………………………………………………..sodium chloride
HRC…………………………………………………….Hawaii Regional Cuisine
UH……………………………………………………….University of Hawaii

Chapter 1. General Introduction

Agriculture

The United States Department of Agriculture (USDA) recognizes that conservation by farmers, ranchers, and forest owners today means thriving and sustainable agriculture for the future. Currently, seventy percent of the nation's land is privately owned (USDA, 2015). Conservation of the nation's private lands allows for healthy soil, water, air, plants, animals and ecosystems while providing productive working lands. Progress in technology and crop yields has made the U.S. among the most productive agricultural producer in the world. For instance, California produces more than half the nation's fresh fruits and is the leading producer of fresh vegetables. More than half of all vegetable production in the U.S. depends on irrigation in California's vast agricultural valleys. However the current drought can cause ripple effects throughout the nation's food system due to general impacts of climate change. Consequently, increased temperature from global warming results in unpredictable weather patterns (rainfall) and more frequent occurrence of extreme weather for instance: increased storms, drought, flooding, and sea level rise. Despite the record revenues (during the drought) in California's agriculture industry (Cooley et al 2015), we need to find more ways to efficiently and sustainably grow food while conserving precious resources like water and land.

Almost 4.6 trillion gallons of water rushes out of Colorado's mountains each year as the winter snow melts. Two-thirds of the water belongs to downstream users (Mexico, California, and 17 other states) while Colorado gets the rest. As the West's population grows, persistent droughts and climate change are expected to limit the supply (Colorado Water Plan, 2015). It's clear that water is a very limited resource in the West. Increased population, demand for energy and food, and the rise of the middle class drive this water scarcity. California's population has grown dramatically coupled with a reduction in supply (very little rain or snowpack) creates an increased demand for water. This consumption of water is outstripping the supply in California's industrial agriculture system. Lack of available water is resulting in staggering losses for the state's farm community. University of California, Davis estimates that the drought prevented farmers from planting 540,000 acres of land this year, costing farmers $1.2 billion and the agriculture economy $2.7 billion as a whole (Howitt et al, 2015). The land fallowed and farmers lost billions of dollars, yet California's agriculture industry posted record revenues in the midst of the drought. The industry was able to maintain high revenues because of a shift to high-value crops, which allows farmers to make more money per gallon of water. These premium crops (nuts and fruits) are also more labor intensive than lower-value crops like alfalfa, offsetting some of the job losses when fields go fallow. There has been a less severe impact on agriculture jobs than some feared. Large, low-value crops like alfalfa tend to require less labor therefore less jobs. Small high-value crops (like pistachios and strawberries) leads to more employment opportunities because tending to smaller crops are more labor intensive (Fox, 2015). Just as California's residents have changed their lifestyles, the state's farm community will need to change their customs of operation as the drought continues. Farmers can switch from flood irrigation to drip or micro-spray irrigation systems, which use less water. Up to forty percent of the water used by some farmers is lost due to inefficient practices such as field flooding. Investing in irrigation controllers that monitor water and soil conditions can deliver water as needed. Some have changed what they plant: reducing production of water-intensive crops such as rice. Nonetheless, innovation and efficiency will be required of agriculture businesses and of ordinary Californians. To cope with the increasing competition for water, management plans for water and land must be implemented by local policy makers, agriculture businesses, farmers and ranchers to keep their ecosystems healthy.

The industrial agricultural system is changing due to the circumstances with a greater emphasis on organic goods. Consumers' demand for organically grown goods has shown double-digit increases over the past decade, an estimated $17.8 billion in 2007, almost 2.5% of total U.S. food sales (Radovich et al., 2009). National and global perspective on organic agriculture has followed demand and shown similar growth. The world value of certified organic crop production was $30 billion in 2005, increasing 14 percent annually from 2000-2005 (Radovich et al., 2009). Organic products have relatively high production costs due to increased labor requirements. However organic and natural foods enjoy a price premium in the market because of consumer interest in healthy, ecologically produced food. Price premiums vary with commodity and in California's case a shift towards high-value crops such as nuts and fruit has allowed farmers to thrive during the drought. California is an example that has brought record revenues into the state by shifting towards high-priced products. Due to consumer demand for organic products the agriculture industry must change practices for efficient food production that incorporate conservation of resources (water) to remain sustainable. As the drought brings challenges to California's agriculture industry it also brings opportunity for farmers not only to grow discreetly but also to adopt alternative methods of growing that minimize water usage.

Aquaculture

The United Nations Food and Agriculture Organization (FAO) estimated that nearly half of the world's consumption of seafood comes from aquaculture. Globally, Asia is the leading continent for aquaculture production. The top producing country in Asia is China (62% of global total), while the U.S. ranks fifteenth in production (FAO, 2015). In 2012, freshwater and marine aquaculture production for the U.S. was estimated to be 594 million pounds with a value of $1.23 billion, a decrease of 17 million pounds (2.8%) in volume and 103 million (7.7%) in value from 2011. Production has declined because of high feed costs and intense competition from imported, frozen fillet products from Asia. Globally the seafood industry depends on extensive aquaculture from China. The U.S. has potential to expand its aquaculture industry sustainably. However, American aquaculture industry faces significant challenges because of opposition developed around concerns over environmental impacts (from intensive aquaculture). There are several efforts underway including Aquaculture Stewardship Council (ASC), which is the world's leading certification and labeling program for aquaculture. They provide strategies to create environmentally and socially responsible aquaculture (ASC, 2016). These initiatives drive a continuous system of improvement, helping the industry shift perception and performance.

Interaction between aquaculture and the environment are diverse and complex. A major issue is the adverse environmental impact of modern aquaculture causing eutrophication because of intensification through increasing use of pelleted feed. Fish growth utilizes only one third of nitrogen in feed, while the rest of the two thirds of nutrients remains in the wastewater. This aquaculture effluent has an adverse impact on the environment because of periodic exchange of enriched fish water into the surrounding environment to improve water quality. Farmers switch to intensive production mainly to increase profits with higher yield due to increased demand in domestic and international markets and availability of new technology. Farmers may choose to maintain a low intensity of production or reduce the intensity of their aquaculture system if these contribute to a more sustainable overall livelihood. Aquaculture in common with other sectors in agriculture should operate within ecological limits to minimize environmental degradation (remain within carrying capacity of the ecosystem).

Tilapia, second only to carp in global aquaculture production, reached 45 million metric tons in 2013 worldwide (Food and Agriculture Organization (FAO) 2015). Aquaculture has great potential to expand sustainably to meet the demand for fish in 2050 as the human population continues to grow before stabilizing at a minimum of 9 billion people (Godfray et al., 2010). There is a clear need to expand aquaculture production in the U.S. Compelling reasons include: the need for additional seafood in the future, offsetting a $9 billion trade deficit in imported seafood products, coastal economic development, expanded employment opportunities, the reality of fully exploited capture fisheries, and enhanced food security. In 2013, the value of tilapia imported into the U.S. exceeded $1 billion, contributing to a global fish production that exceeded the cost of beef in 2012 (FAO 2012). Primary issues for the expansion of domestic aquaculture include: availability of freshwater resources, competition with imported products, and a supportive regulatory process for marine aquaculture. Solutions for environmentally sustainable aquaculture are required to meet the increase in demand for aquatic food. This is more likely to be met through various combinations of technological developments, improvements in existing technology, better management practices, and better site selection so that aquaculture remains within carrying capacity of ecosystems. It is noted in NOAA’s ten-year plan for marine aquaculture that by 2025 American aquaculture production could more than double, adding one million tons of production and creating 75,000 new jobs (NOAA, 2007).

1.1.c. Aquaponics

Aquaculture (fish farming) and hydroponics (growing plants without soil) are the building blocks of aquaponics. It is a soil-less natural process that can be found in lakes, ponds and rivers. Fish waste utilized as fertilizer for crops is an ancient practice. The most well-known examples are the “stationary islands” set up in shallow lakes in central America (e.g., Aztec’s Chinampas 1150-1350 BC) (Turcois, 2014), and the introduction of fish paddy rice fields in Southeast Asia about 1500 years ago (Goddek, 2015). Even the ancient Hawaiians demonstrated this practice in freshwater taro fishponds (Loko I`a kalo). The most studied example was set up at the University of Virgin Islands in 1980 by Dr. James Rackocy (Rakocy, 1989) also known as the Father of Modern Aquaponics. Rakocy was effectively the first person to develop a fully functional commercial scale aquaponics system.

Aquaponics is an integrated system that combines elements of recirculating aquaculture and hydroponic. In aquaponics fish are raised at high density in a relatively small volume of water in a recirculating aquaculture system (RAS). The nutrient-rich water (effluent) that is produced by raising fish provides a source of natural fertilizer to nourish the plants. Bacteria in the system break the waste down into nitrate allowing the plants to utilize nitrogen in this form. When the plants take up the nutrients, the roots purify the water that the fish live in. This creates a sustainable micro ecosystem where both the plants and fish can efficiently thrive in a symbiotic environment. Aquaponics is growing in popularity because it solves many of the problems that strike traditional soil-based growers worldwide. This is particularly the case with increased emphasis as regards to water use, environmentally friendly produce and the concern regarding depreciating fish stocks. Sustainable agriculture is defined as a process that does not deplete any essential non-renewable resources in order to sustain the agricultural practices (Feenstra et al, 2016). Tyson (2007) reported that aquaponics fits closely with the definition of sustainable agriculture because it “enhances environmental quality” by producing crops with practices that minimize water and nutrient waste discharges into the environment. Aquaponics allows intensive aquaculture to be eco-friendly by reducing the environmental impact caused by the effluent as polluted fish water is cleaned up instead of being released into the environment. Thus, aquaponics is a sustainable method of food production because it recycles nutrients, mimicking natural ecosystems.

Aquaponics has been generating increasing interest from scientists and potential commercial operators, as few successful commercial farms exist today. However, aquaponics is economically challenging. According to Tokunaga et al. (2015), in Hawaii commercial aquaponics cannot tolerate low prices for vegetables, low system biological performance, high capital expenses, or high operational expenses and still remain profitable. RAS offer the potential for relatively minimal environmental discharge but systems are complex with high capital and operating costs. Still, aquaponics is viable depending on location and climate. Aquaponics is suitable for environments with limited land and water because it produces about three to six times the vegetables (Resh, 2004) and uses about 1% of the freshwater used by traditional aquaculture (Rakocy, 1989). In most Pacific Islands, vegetables are very expensive because they must be imported by airplane or boat. Nonetheless, aquaponics is commercially promising as an organic food crop production system in Hawaii (Tokunaga et al. 2015).

Advantages and Disadvantages of Aquaponics: De-nitrification, Water controversy, Food security.

Summary

Aquaponics is a promising sustainable food production method; as it resembles a natural small-scale ecosystem and is designed to close the nutrient cycles. Negative components of hydroponic and RAS become advantages when integrated into aquaponic systems. Benefits that aquaponic techniques offer include: efficient crop growth, low resource requirements, and high production on marginal agricultural lands. However, not all components of the system are beneficial. For example, if not properly maintained, water conservation is an issue and nitrous oxide can be emitted into the atmosphere (Hu et al, 2015). The aquaponics concept is promising to contribute to global and urban sustainable food production while simultaneously diminishing pollution and the strain on non-renewable resources.

Advantages and Disadvantages

Hydroponics and aquaculture independently have certain negative aspects. Hydroponics requires expensive nutrients from nonrenewable resources to feed the plants. A considerable amount of macro- and micronutrients are required from industrial mining origin (Tyson, 2004), which leads to high-energy consumption for production and transport. In non-recirculating hydroponic systems, periodic flushing of the nutrient rich water leads to high water consumption as well as waste disposal issues such as surface and groundwater pollution (Beyers, 2004). RAS (or intensive aquaculture) also must remove excess nutrients from the system. RAS is defined as: large quantities of fish that are raised in a relatively small volume of water that is constantly recycled. A portion of the effluent wastewater is removed daily and replaced with freshwater to improve water quality. A recent study by a commercial hydroponic greenhouse in Belgium reported that the RAS water could only supply about 25% of the nitrogen, phosphorus and potassium needed by the plants (Timmons et al, 2002). Furthermore, reusing RAS wastewater to save on fertilizer costs in hydroponics was hardly an issue, as the cost of artificial fertilizers only comprised 2% of the total production cost in hydroponics (Edwards, 2015). Fertilizer cost may be cheaper, however access to these resources (inorganic fertilizers) are restricted and thus unsuitable in the sense of true sustainability. In terms of sustainability, both phosphorus and potassium are major components of agricultural fertilizers, and like oil, they are non-renewable resources. Therefore, increasing usage and depletion of these minerals without reuse or recapture has a negative impact to their future supply. This use of finite resources such as fertilizers and freshwater will have dramatic consequences for global food security (Edwards, 2015). Thus, aquaculture and hydroponics taint the environment due to the discharge of polluted water into the surrounding environment to enhance water quality. Moreover the reliance on non-renewable resources in unsustainable.

While recirculating aquaculture and hydroponics are both efficient methods of producing fish and vegetables, respectively, when combining the two, the negative aspects previously described are converted into positives. For example, aquaponics allows efficient nutrient cycling and water conservation. Excess nutrients do not need to be removed through periodical exchange with fresh water as practiced in aquaculture systems. The advantages of linking fish and plant culture together are shared startup, operating and infrastructure costs, fish waste nutrient removal by plants, reduced water usage, and increased profit potential by producing two cash crops (Rakocy, 1999; Timmons et al, 2002). Vegetable production predominates in aquaponic systems, which may be an advantage if there is a good market for the vegetable crop (Edwards, 2015). According to Tokunaga et Al (2015), most of the profit comes from plant production because fish, especially tilapia, take time to grow. Once hydroponic systems are integrated with aquaculture, crop production is considered organic and there is a price premium for organic produce in the market. Nonetheless, within this synergistic interaction, the respective ecological weaknesses of aquaculture and hydroponics are converted into strengths.

There are other ways to produce fish and vegetables efficiently and more economically. It may be affordable with the need for less management skills to produce hydroponic vegetables in inexpensive plant crop systems fertilized with inorganic fertilizers rather than through integration with a fish recirculating system. Still this use of minerals from finite sources and ineffective water usage is not sustainable. Depending on location and system design there is potential for aquaponics in arid climates and/or niche markets where water is especially scarce, and consumers are willing to pay a higher price for high-quality fish and vegetables. In places such as islands as well as urban cities (where the industry depends on imports) land and water are limited. These environments have potential for aquaponics. Nonetheless there are various advantages and disadvantages, but in certain settings (island/cities) the advantages outweigh the disadvantages. Therefore aquaponics is a way to be sustainable under the circumstances.

To be sustainable we must meet the needs of the present without compromising land and natural resources for future generation. Aquaponics is one way to accomplish this.

Disadvantage: De-nitrification

Aquaponics offer the potential for relatively minimal environmental discharge but systems are complex (Edwards, 2015). Aquaponic systems have high capital and operating costs, high-energy inputs with higher greenhouse gas emissions per unit of production than pond and cage culture. A major disadvantage of aquaponics is de-nitrification, which emits nitrous oxide (N_2O) into the atmosphere (Tokunaga et al, 2015). The analysis of McGee's (2015) study finds that the rise in certified organic production in the U.S. is not correlated with declines in greenhouse gas emissions derived specifically from agricultural production, and is associated positively overall to agricultural greenhouse gas emissions.

Human activities such as agriculture, fossil fuel combustion, wastewater management, and industrial processes are increasing the amount of N_2O in the atmosphere (EPA 2015). According to Hu et al. (2015) aquaponics has high nitrogen utilization efficiency (NUE). Nitrogen is a vital element for all living organisms and protein-rich fish feed is the major source of nitrogen for fish cultivation. In aquaculture system, about 25% of the nitrogen input is harvested through fish biomass, and over 70% is excreted into the surrounding environment in the form of ammonia (Hargreaves, 1998). This ammonia is converted to nitrate (by bacteria) and absorbed by the plants. Nitrogen takes on a variety of chemical forms throughout the nitrogen cycle, including N_2O. When there are low nitrate levels coupled with high amounts of fish feed de-nitrification occurs (Ako, 2014). De-nitrification is the conversion of fertilizer nitrate to nitrogen gas through a series of intermediate gaseous nitrogen oxide products that is released into the atmosphere. Under anaerobic conditions (no oxygen) denitrifying bacteria convert nitrate (NO_{3-}) to nitrogen gas (N_2). The application of nitrogen-based fertilizers also accounts for N_2O emissions in aquaponics. According to the EPA (2015), the impact of 1 pound of N_2O on warming the atmosphere is almost 300 times that of 1 pound of carbon dioxide. Nitrous oxide emissions occur naturally through many sources associated with the nitrogen cycle, which is the natural circulation of nitrogen among the atmosphere, plants, animals, and microorganisms that live in soils and the oceans. To minimize de-nitrification in aquaponics as a rule of thumb nitrate levels should be around 45 mg/L (Ako, 2014) and by reducing nitrogen-based fertilizer application.

Agriculture generates one third of all man-made greenhouse gas emissions. Aquaponics will not help reduce these emissions but this can be minimized if systems are maintained properly. Despite this weakness, aquaponics is more cost-effective and efficient than traditional farming techniques. While cost is high because aquaponics merges components of aquaculture and hydroponics, overall it would be less expensive.

Controversy: water use efficiency

A critical challenge for the early 21st century is the resolution of the water crisis, increasing scarcity, and quality of water in the near future, with less water available for agriculture and aquaculture (Molden, 2007). One-kg of fish bred in semi-intensive and extensive aquaculture systems requires a range of 2500-375,000 L (Al-Hafedh, 2003). In recirculating aquaculture systems water usage is below 100 L/kg of fish produced (Martins, 2010). Agriculture uses about 70 percent of the world's fresh water, and shortages will have a huge impact on food security.

In aquaponic systems water recirculates. Runoff water that is not taken up by plants is recaptured and reused, in contrast to traditional, soil-based agriculture. Water is continuously salvaged but depending on certain conditions (high temperature) typical water loss occurs via evapotranspiration. Evapotranspiration is inevitable. It is a function of living plants where water is evaporated through leaf tissue. Hu et al (2015) concluded that with a 5% daily water exchange, aquaponics does not conserve water especially for plants with large exposed leaf surface. To minimize this as efficiently and biologically possible, temperature should be within range of the specific crop. Covering systems (tanks and plants) adequately with black shade cloth can improve the situation. So that plants do not use this mechanism (evapotranspiration) to cool off in high temperatures during the day. Aquaponics conserves water relative to soil-based agriculture especially when growing the same highly evaporative plant species. Certainly water is conserved compared to traditional agriculture and aquaculture. However the issue of evaporation prevents the conservation of water on a daily basis and thus prevents aquaponics from being sustainable unless a preventative mechanism is used.

Advantage: Food security

Aquaponic's role for food security is valuable. Offering economic prosperity in remote communities in third world nations as well as household consumption in developed nations. It is relevant because the global population now exceeds 7.2 billion and is continuously growing. By 2030, global population will reach 8 billion people, with more than 75% living in urban areas (Goddek, 2015). Urban population growth will require an increasing demand for animal protein (Alexandratos, 2012), as global calorie demand will increase 50 percent. However, raising and fluctuating energy and oil costs, climate change and pollution challenge the future of conventional farming, including intensive animal protein production, in meeting this demand. Aquaponics can compensate existing sustainable deficits in agricultural food systems.

If access to fresh produce is disrupted for whatever reason it can be very beneficial to have your own source of fresh and healthy food. This allows household food security that is fully under your control and independent of any problems in the food distribution system. Consequently, in communities where food is scarce and difficult to acquire, small-scale aquaponics can help at-risk communities to find ways to produce healthy food for consumption and for income generation. Moreover access to food in remote indigenous communities is poor. Along with the added stress of supply being intermittent as a result of many factors related to remoteness and lack of storage. When perishables such as fruit and vegetables do reach the communities it is often of low quality and in small variety, depending on what can be stored and the most 'economic' to transport. Aquaponics has the potential to enhance food security, but there are several concerns that need to be addressed. Resource limitations including access to electricity and constrained freshwater supplies also add to these challenges. With the correct support structure aquaponics could provide opportunities for smallholder farmers and increase food availability especially in developing nations with infertile lands and harsh growing conditions. Economic growth is a key success factor in providing opportunities for improving the livelihoods of these communities.

For remote communities to become truly sustainable, steps should be taken to increase food security through innovation and creativity for better health, wealth and wellbeing. Aquaponics allows economic growth for societies with resource limitations while providing a reliable source for produce. Enhancing the productivity and incomes of smallholder family farmers is key to progress (FAO, 2016).

Global challenges and Opportunities: aquaponics takes pressure off global challenges.

A global disaster: overfishing depleted fish stocks

Eighty percent of the world's oceans are fully-or overexploited, depleted or in a state of collapse. One hundred million tons of fish are consumed worldwide each year, providing 2.5 billion people with at least 20% of their average per capita animal protein intake (FAO, 2012). Fish is one of the most efficient animal protein producers. Since fish demand is increasing while the fishing grounds are overexploited (MEA, 2005), aquaculture is the fastest growing sector of world food production (FAO, 2015). Adverse effects of this development include high water consumption in case of conventional fish protein production (EPI, 2008), and release of up to 80% of N and 85% of P per kg of fish feed (Van Rijin, 2013; Schneider et al., 2005) into the environment. This causes the loss of valuable nutrients, resulting in eutrophication in rivers, lakes and coastal waters, and excessive productivity leading to vast dead zones in the oceans (Dybas, 2005). The influence of human activity on the oceans has expanded from direct to indirect interference via environmental changes on land and in the atmosphere.

According to the Western Pacific Regional Fishery Management Council, Hawaii only produces about one to two percent of the world's bigeye tuna supply (Kaleo, 2015).

Despite its small impact, Hawaii holds a big responsibility as a representative of the U.S., the world's fourth largest producer of fish. On a local level, Hawaii's total aquaculture sales in 2011 were valued at $40 million, increasing $10 million from 2010 (HDOA, 2016). Producing more seafood locally is in line with the State's food self-sufficiency initiative and helps build a strong regional food system in Hawaii. However, overexploitation of fisheries is a worldwide problem that has led to the collapse of much of the fish stock; in some areas there are no fish to catch. At this rate our oceans could be fishless by the year 2050, according to a 2010 UN report. However, recently a global assessment of fish biomass concluded that predatory fish in the world oceans has declined by two-thirds and continues to decline, with 54% occurring in the last 40 years (Christensen, 2015). As a consequence of overfishing, our future fish supply will predominantly be small prey fish (such as sardines and anchovies) due to predation release. Beyond ecological consequences, collapsed fishing stocks means loss of jobs. Overfishing has not only compromised people's livelihoods and health of our oceans but has wiped out entire species of fish we eat. In the end, we simply need to reduce our consumption of fish and realize that the resources of our planet are not infinite.

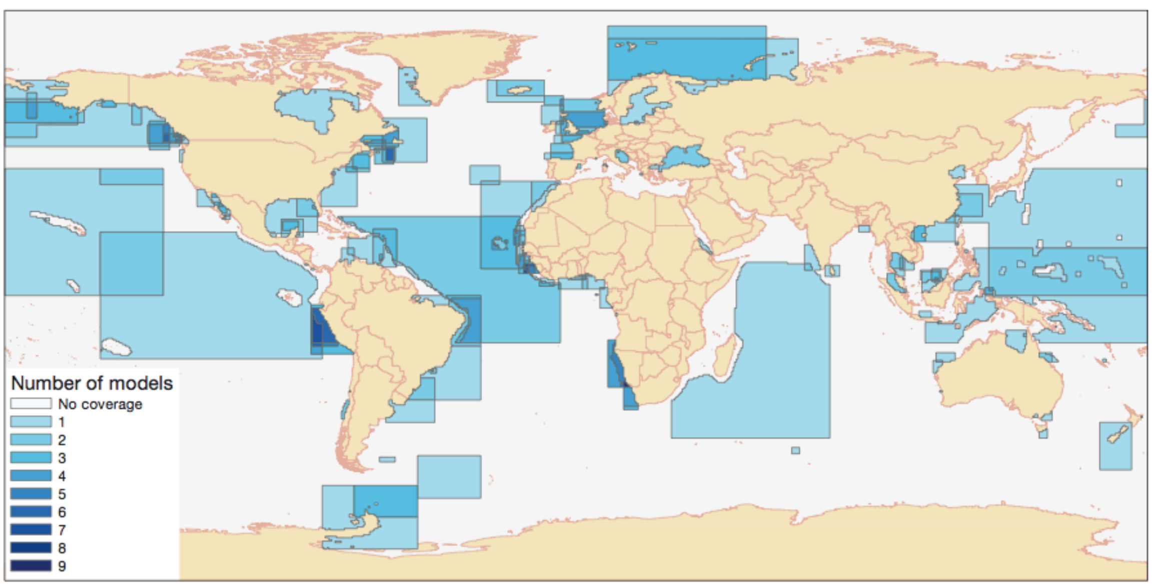


Figure 1: Fish biomass decline in the ocean (Christensen et al. 2015): Spatial distribution of the ecosystem models illustrating the wide global coverage. Color density is indicative of models at each location.

Water Crisis: why it's so important to conserve water.

Another resource that becomes increasingly scarce is freshwater. Water is too valuable to waste. Finite freshwater resources are under increasing stress from population growth, pollution and the demands of agricultural and industrial uses. Water conservation is a topic of increasing concern, especially in the drought-ridden west. Drought conditions and lack of water impacts agriculturalists, municipalities, industries, and individuals. Aquaponics is one approach that can reduce water loss, increase water use efficiency, and use water more sustainably in agriculture.

On average, global agriculture uses around 70% of the available freshwater resources. In arid climate zones such as the Middle East and North Africa, the agricultural water consumption can even be up to 90% (FAO, 2005). Agricultural flood irrigation in large fields loses water to simple evaporation, runoff, and dispersion beyond the reach of plant roots. Not only is the farmer's hard-earned money draining away into the ground but, also, as the water drains away, it collects fertilizers and chemicals, leaching into the groundwater (drinking water). The excess minerals flow downstream into rivers and oceans destroying the aquatic life. The agriculture industry is changing practices to be more water-efficient, but even the best drip irrigation only cuts flood irrigation losses sparsely.

Aquaponics is increasing in popularity because it resolves issues that strike conventional soil-based growers worldwide. Compared to conventional agriculture, aquaponics uses less than 10% of water, depending on the climatic conditions (Berstein, 2012) and system design. Water use efficiency in an aquaponics system is drastically lower than that of traditional agriculture and other leading competitors. This not only lowers water bills, but allows water to be used more sustainably. Modern aquaponics not only recycles water and but also recycles nutrients within the system. Within the cycle waste in one part of the system is utilized as a resource in another. Pollution is drastically reduced because the water and the wastes contained are recycled instead of being dumped into the ground water.

Water is a scarce commodity. Without clean drinking water humans cannot survive. Aquaponics can reduce freshwater depletion associated with irrigation while guaranteeing safe encouraging sustainable farming and food production practices. However, system-related water losses do occur through plant transpiration. According to Hu et al (2015), evapotranspiration from plant leaves prevents water conservation. Yet, with proper care and management water input can be minimized.

An increasing number of countries are facing economic and physical water scarcity, leading to a growing incapability in feeding their people (WWAP, 2012). Already, some 700 million people worldwide suffer from water scarcity, but that number is expected to swell to 1.8 billion in just ten years (Talbot, 2014). After water conservation, recycling, and even treating and reusing sewage, seawater desalinization is an option of last resort. This process uses reverse osmosis to transform saltwater into potable water. Desalinization is one of the most expensive sources of freshwater because it takes much energy to push water through the commercial membranes. Recently San Diego county government decided to build the largest seawater desalinization plant in the Western Hemisphere, at a cost of $1 billion (Talbot, 2014).

Organic Agriculture

Consumers' demand for organic products was estimated at $17.8 billion in 2007, almost 2.5 percent of total U.S. food sales (Radovich et al., 2009). A broader range of consumers has been buying more organic products than ever before. Production statistics data indicate that organic agricultural production in Hawaii has followed the national and international trends of sustained growth (Radovich et al., 2009). Organic foods now occupy prominent shelf space in the produce and dairy aisles of most mainstream U.S. food retailers (Dimitri et al 2009). There is interest among growers in producing organically, however growing organically is not a requirement. As organic agriculture production continues to grow, policy makers are facilitating the efforts of producers that are interested in expanding in this area. As the trend follows demand, prices of organic products are generally decreasing as the supply of organic produce increases due to competition.

At the national level, organic growers have indicated that they face a number of challenges. Shifting to an organic production requires high managerial costs with higher risks. A lack of technical knowledge about organic growing methods and requirements to become certified organic is also another challenge. Locally, growers in Hawaii cannot capture economies of scale in marketing or production (Radovich et al., 2009). Growers should operate more efficiently since cost advantages are obtained by shifting to organic. Due to scale of larger operation as quantity of production increases, the average cost of each product decreases. However this does not happen for small conventional farmers in Hawaii due to a lack of distribution and related infrastructure.

For organic food crop systems crucial infrastructure matters include water, land, and labor. To address water issues: improvement of existing irrigation systems and progression toward a sustainable water plan are a concern. The high cost of land and lack of availability of long-term leases is also a challenge for organic producers. To become certified, organic growers need land that has not been exposed to restricted substances for three years, and they must demonstrate active stewardship of soil and other resources (Radovich et al., 2009). This requires large investments in labor and other resources, which makes long-term land even more important in this sector compared to conventional growing systems. Conventional producers often express concern about high costs and low returns associated with agriculture in Hawai'i due to high cost of imports. Organic food crop systems have the opportunity to earn immense profit because of the consumers' demand for eco-friendly farming practices. However, organic products are more costly due to the increased labor requirements (Kremen, 2006). Allowing visitor stays on farms and providing hospitality training for hosts would aid growers. For example, WWOOF: a worldwide opportunity on organic farms is a labor exchange program; individuals volunteer on an organic farm and experience living an organic and sustainable lifestyle. Allowing growers and workers to reside on the farm would also be one deterrent to theft. However expense for these workers may increase over time due to food and living accommodations; thus local residents are another source of labor. Lastly, aquaponics eases the infrastructure challenges related to conventional organic agriculture such as

water, land, and labor. As an alternative form of food production system, aquaponics allows producers to be organic without land restrictions while conserving water. However in spite of the advantages, the USDA is considering banning aquaponics and organic hydroponic (hydroponics using organic nutrient sources) from organic farming.
McGee argues that recent USDA certification of organic farming has divided the organic market, where one form of agriculture is sustainable compared to conventional agriculture while the other works to increase the economic accessibility of organic farming by weakening practice standards that would reduce agriculture greenhouse gas output (McGee 2015).

Urbanization

Land is a challenge in conventional agriculture, and yet aquaponics can be developed in any place. It has the added benefit of an organic production without the use of fertile land. This also allows an opportunity for urban farming with short supply chains. Aquaponic systems can be set up almost anywhere and have the potential to urbanize food production. For instance, aquaponic plants can be implemented in old industrial neglected buildings with the advantages of re-establishing a sustainable activity without increasing urbanization pressure on land. Roof gardens would be another possibility, allowing for the conservation of space in urban areas. Another important aspect is minimizing the distance between the producer and consumer. The longer the supply chain, the more transport, packaging, conservation and labor needed, leading to substantial decreases of resources and energy. Shortening and simplifying the food supply chains can drastically diminish their environmental impacts, while providing cities with fresher products. Allowing the consumer to clearly identify his food origin.

Economic Viability

Operating in a resource limited world. Energy cost and fertilizer prices are constantly rising and governmental policies encourage the reduction of emitted pollution. Both phosphorous and potassium are major components of agricultural fertilizers, and like oil, they are non-renewable resources. Therefore, mineral recycling is crucial in order to remain sustainable and to avoid dramatic consequences for food security. Although the highest financial profit margin in aquaponics has been shown with leafy greens, it is still necessary to determine the purpose and the scale of the systems before building them. The needs in terms of local food supply might differ from profit-oriented approaches and from country to country. For each location, different measures are needed to ensure that each system will have a suitable energy source all year round to provide stable conditions for fish and plants.

Although preliminary research has shown that developed aquaponics is not fully realized in cost effectiveness, Tokunaga et al conclude that commercial aquaponics is economically efficient. As long as they are certified organic and implement renewable energy. However the commercial development of socially, ecologically, and environmentally sustainable aquaponic systems confronts several technical challenges that need to be addressed further: (1) improved nutrient solubilization and recovery for a better use of the nutrient input and reducing extra-mineral addition e.g., phosphorous recycling; (2) adapted pest management; (3) reduce water consumption by limiting the need for water exchange; (4) use of alternative energy resources for various climates; and (5) innovative pH stabilization methods.

Aquaponics in Hawaii

Aquaponics is an agriculture system that assists Hawaii in becoming more self-sufficient within the island. There is potential for aquaponics in Hawaii because of the state's dependency on imports, current operations within the state, and consumers' demand for locally sourced goods. From 2000 to 2009, Hawaii imported on average 317,000 kg of tilapia annually (Loke et al. 2012) and 89% of the total lettuce consumed in 2007 (Tokunaga, 2015). Hawaii heavily relies on imports for the majority of its food consumption. The State's reliance on imported lettuce is still significant and there is room for aquaponics to close the gap between local production and imports from the mainland.

Hawaii is a suitable location for aquaponic production. The aquaponic farms that are currently in-operation in Oahu are relatively small. Yet, they confirm the economic feasibility of the aquaponic industry in Hawaii. According to Tokunaga et al. (2015), small-scale commercial aquaponics is profitable, which suggest potential for commercial aquaponics to supply vegetable and fish to the local market. This study finds a scale effect: the bigger the system, the higher the rate of return. Aquaponics is more profitable than stand-alone hydroponics by assuming equivalent production volume and requirements. Thus, aquaponics is a viable food production technology.

Furthermore, consumers in Hawaii make conscious decisions to purchase locally produced products. Vegetable and fish are sold at the market on a regular basis throughout the year. There is potential for aquaponics to become a major supplier of vegetables and fish in Hawaii's market to meet its increasing demand. This can help produce food for the local market efficiently enough to replace most imports such as lettuce and other perishables. Aquaponics is responding to diverse ecological and social challenges, which point to the importance to focus on efficient and sustainable forms of agricultural production.

Chapter 2.

Background and Significance

In an effort to combat climate change, many farmers around the country are developing innovative methods of sustainable growing, such as aquaponics. This combination of fish and soilless plant farming is increasing in popularity as a more eco-friendly method of food production. According to the United States Department of Agriculture, agriculture is a major user of ground and surface water in the U.S., accounting for approximately 80% of the nation's consumptive water use (USDA, 2015). Modern agriculture as well as aquaculture wastes too much water, thus there is a need to adopt new growing techniques that require less resources. As a sustainable solution for fish and water consumption, both fishers and farmers can benefit from aquaponics – producing food while using fewer resources.

Aquaponics combines aquaculture (fish farming) and hydroponic (growing plants without soil). More specifically it is defined as a system of aquaculture in which waste produced by farmed fish or other aquatic animals supplies nutrients for plants grown hydroponically. Fish manure provides a natural fertilizer for the plants, while they purify the water for the fish. This symbiotic environment resembles natural ecosystems. Aquaponics is ideal for homegrown food production because it makes gardening available to a wide range of people who may have found it difficult due to urban environments, disabled etc. According to Tokunaga et al, commercially aquaponics is economically challenging however it also fits the criteria for sustainable agriculture.

Aquaculture is the fastest growing sector of world food production (The Fish Site, 2014; Rohana, 2005). However according to the figures published by the UN *Food and Agriculture Organization* 80% of the world's oceans are fully-or over-exploited, depleted, or in a state of collapse (Subasinghe, 2005). Since human population is on the rise, fish demand is increasing while the fishing grounds are overexploited. The future of conventional farming in meeting this demand is challenged by rising energy costs, climate change, and pollution. The concept of aquaponics is promising to contribute to global and urban sustainable food production while diminishing pollution and the need for nonrenewable resources. Especially regarding water use, environmentally friendly produce, and the concern regarding depreciating fish stocks. Thus, there is a necessity to integrate existing sustainable practices in agricultural systems to promote global food security.

By 2025, 1.8 billion people will experience absolute water scarcity, and two thirds of the world will be living under water-stressed conditions (UNFAO, 2013). Aquaponics is one way to address this global challenge as it uses less than 10% of the water required for conventional agriculture. However, over time the pH of the water becomes acidic because of the process of nitrification (converts fish waste-ammonia-to plant food-nitrate). This acidic environment is toxic to the fish, plants and bacteria in the system.

This experiment will determine various buffer applications to remediate pH. Buffering capacity is defined as the ability to maintain pH consistently for a designated period of time. To understand the treatment design, the distinction between a base and a buffer must be understood. A base or buffer is a substance capable of reacting with an acid; only a buffer can maintain constant pH. For example the powder form of potassium carbonate is a base with an immediate effect on pH once applied. After the base is absorbed by the aquaponic system the pH returns back to the original acidic condition within 48 hours (M. Khawaja, observation, February 22, 2014). This temporary base causes stress to the system, ultimately inhibiting plant and fish growth. On the other hand, oyster shells are a buffer because of its larger surface area. Compared to the surface area of powder, 1-mm oyster chips cause a steady increase in pH towards neutral pH (allowing the system to slowly absorb the buffering component). Due to the surface area of the particular treatment the pH will be adjusted for varying lengths (days to months).

The goal of the proposed study is to understand the effects of buffers and fertilizer application in aquaponics and its influence on crop yield and quality in the tropics. This knowledge will primarily increase awareness for commercial farmers and the local community as well. This study also allows researchers and agriculture professionals to resolve gaps in our knowledge about aquaponic agriculture and food security issues. More importantly, this will help to decrease reliance on imported food sources, especially on the heavily developed island of Oahu. To achieve this goal, the following objectives and hypothesis are developed as described below.

Experimental Design

Purpose: Determine treatment effect in aquaponics in relation to water quality, fish growth, plant yield, and capsaicinoid content.

Trial	Activity	Outcome
1. Preliminary Study in aquaponics	▪ Establish water quality parameters ▪ Monitor pH	Determine how long it takes pH to become acidic.
2. Aquaponic Experiment: remediate pH	▪ Apply treatment ▪ Measure: water quality, fish growth, crop yield	Compare buffering capacity and understand treatment effect
3. Capsaicinoid analysis of Hawaiian chili	▪ Sample prep ▪ Extraction ▪ HPLC run	Find strategies that manipulate yield and spiciness of peppers (farmer profitability)

Table 1 Experimental design outline.

Objective 1: Establish baseline water quality criteria for aquaponic experiment.
Hypothesis: If nitrification continues in aquaponics then pH becomes acidic (pH below 5.5).

Rationale: Aquaculture is the fastest growing food sector since the 1980s and accounted for almost half of the global seafood consumption in 2012. To meet the demand for aquatic products, aquaculture is bound to expand. However, aquaculture is a high-polluting industry. On average only 25% of nutrients are recovered by the fish and the rest is discharged into the surrounding environment (Hue et al, 2015). Thus aquaponics is considered to have the potential to solve the problems of aquaculture. Aquaponics is another form of farming that links hydroponics and aquaculture; minimizing the pollution that is caused by traditional aquaculture. With the benefits of aquaponics, over time nitrification causes the pH to become acidic, which depends on multiple factors such as:

feed input, nitrification turnover, and water flow rate. This trial is conducted to determine how long it takes for pH to become acidic.

Outcome: The goal of the preliminary trial is to determine how long it takes for system water to become acidic. Weekly water samples are used to determine ammonia, nitrite, and nitrate concentration using API water quality kit. Water quality parameters such as conductivity, dissolved oxygen, temperature, and pH are measured with meters that must be calibrated before each use. Monitoring water quality measurements weekly is crucial for the experiment in order to keep everything on track (mimic sensors manually every week). Once duration is determined mechanisms to control pH will be tested for the next trial.

Figure 2 (left) Each fish tank is connected to two grow beds with three pepper plants in each bed. Experimental unit is the fish tank, consisting of 1-kg tilapia that are fed 2.5-grams of commercial trout feed daily.

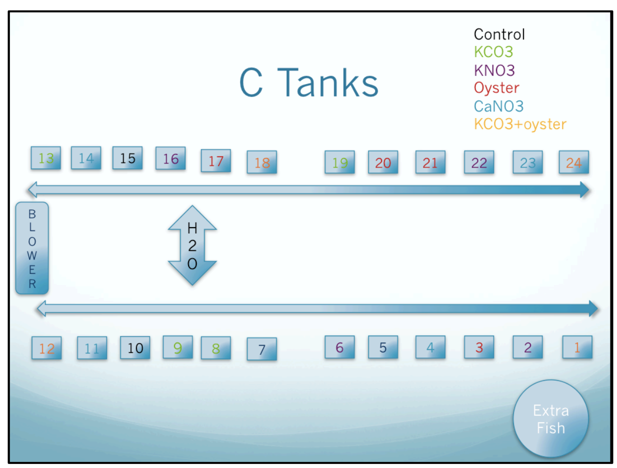


Figure 3 (right) Schematic of system setup 2x24 individual systems. Four replicates for each treatment with a total of 24 systems.

Figure 4 (above) Peppers grown in cinder grow beds that lay above a 400-liter fish tank.

Objective 2: To remediate pH in aquaponic systems to compare buffering capacity and to understand treatment effect within the system components (fish, plants, bacteria).

Hypothesis 2: If pH becomes acidic, then treatment application remediates pH (pH 6-7).

Rationale: Bacteria are the building blocks of aquaponics. They convert the fish waste into fertilizer in a timely manner, which is absorbed by the plants. This process of nitrification prevents ammonia, nitrite, and nitrate from building up in the system. However, under acidic pH this creates an unfavorable environment for nitrifying bacteria (Tyson, 2004). This imbalance in the system can lead to juvenile fish death because of accumulation of ammonia, nitrite or nitrate. If these levels are not within normal levels for prolonged period of time adult fish growth is reduced because of high nitrogen content in the system from both treatment application and the feed.

A crucial point in aquaponic systems is pH stabilization, which is critical to all living organisms within a cycling system. Naturally over time, the pH of the water becomes acidic due to the biochemical process of nitrification, resulting in a toxic acidic environment. A limitation of aquaponics is how to remediate the pH, more specifically what types of buffer and how much to use, or how long they last in the system. One approach to counteract the pH is the addition of nutritional supplementation: with the addition of carbonate, bi-carbonate or hydroxide to the system; adjusting the pH temporarily. Treatments are categorized as buffers (treatments) and fertilizers (controls) in order to understand nutritional aspects of buffer treatments. Fertilizer treatments are used as control to add similar ions to each. The treatments include:

Buffers

1.) Potassium carbonate K_2CO_3

2.) Calcium carbonate $CaCO_3$ (oyster shells)

3.) Potassium carbonate + oyster shells

Controls

4.) Calcium nitrate $Ca(NO_3)_2$

5.) Potassium nitrate KNO_3

6.) Absolute Control (only water)

A completely randomized design is used for the experimental design, which allows complete flexibility with the number of treatments and replicates. Treatments for the experiment were tested on each fish tank; six treatments are tested on a total of 24 systems with four replicates.

Treatment schedule is designed so that the pH will be stable and neutral. The powder form of the base potassium carbonate (KCO_3) remediates the pH temporarily and returns to original acidic pH within 48 hours. Therefore application rate of potassium carbonate is three times weekly in order to maintain neutral (pH 7). Due to the solubility of the base compounds, they are applied thrice weekly along with the nitrate treatments in order to compensate for the fertilizer effect. Calcium treatments applied daily (sits in a mesh bag inside fish tank).

Outcome: The goal of the treatment is to maintain pH 7 for the system (which includes plants, fish, and bacteria) to function efficiently in a symbiotic environment. It can be predicted that potassium nitrate will have negative impacts on fish growth because of high nitrate concentration in the system. High nitrate-nitrogen content is toxic for fish, inhibiting growth. This experiment will allow researchers to make recommendations to aquaponic farmers in the community about long-term pH adjustment for each component in the system. This will increase awareness for aquaponic community locally and globally including commercials farmers and backyard practitionars on how to develop mechanisms to ensure proper pH through access to publications, workshops, and free brochures.

Objective 3: Quantify capsaicinoid content between treatments for crop quality analysis (pungency) in *Capsicum frutescens* (Hawaiian chili pepper).

Hypothesis 3: If treatment application is applied, then the capsaicinoid content increases.

Rationale: Chili pepper plants were cultivated to measure plant health and productivity in an aquaponic setting. *Capsicum frutescens* also known as Hawaiian chili pepper was grown to see if there is a difference in capsaicin content between the treatments. Capsaicin is a phytochemical (secondary metabolite) produced by peppers, which was used as a deterrent against certain animals. In the experiment this characteristic was exploited as an indicator of plant stress. Capsaicin is a stress compound found in the pericarp of the fruit. If plants are stressed due to environmental conditions such as acidic pH, there will be a higher than normal concentration of capsaicin in the chili. This will help determine which treatment is best for the plant.

Outcome: Crop is harvested weekly and samples are collected to quantify capsaicin content in the pepper. The samples are freeze-dried with liquid nitrogen and then dried for 48-hrs. Extraction technique and HPLC conditions are performed as reported in Collins et al. (1995). Specific effects of the treatments on capsaicin content will be compared for fertilizer effect (nutritional benefit), yielding in varying concentrations of capsaicin. This experiment primarily will determine which treatment has the best effect on the entire system in order to make a recommendation to aquaponic farmers in the community. Second, to see if there is an effect on the spiciness of the pepper to make correlations between treatment and plant quality. Depending on the farmer and his interests he can make informed decisions about proper pH balance specific for his needs. For example, environmental conditions can be exploited for plant growth to achieve a desired capsaicin concentration for mild, medium or hot varieties of chilies. This would allow farmers to gain profit by targeting niche markets in demand for various levels of heat.

Table 2: Experimental Timeline (maintenance)

Daily Tasks	Weekly Tasks	Monthly Tasks
Feed fish	Apply treatments	Plant seedlings
Clean grow beds (algae, weed)	Measure water quality parameters	Transplant
Check pipes for leaks	Skills workshop	Harvest

Chapter 3

Preliminary Trial in Aquaponics

Introduction

Principles of aquaponics

In aquaponics fish, plants, and bacteria co-inhabit the same ecosystem. The system integrates aquaculture and hydroponics, where fish waste is taken up as a nutrient source for plants grown in soilless culture. This treatment of wastewater with aquaponic plants is one of many phytoremediation strategies. For example, in a wastewater treatment plant a confluence community exists in which microbe speed the removal of organic substances in the wastewater with high biochemical oxygen demand (Tyson et al. 2007). For bacteria in an aquaponic system, ammonia is the start of the food chain that leads to the production of nitrates. In all, nutrient-rich water from the fish culture is pumped into hydroponic beds to fertilize the plants. Plants absorb the nutrients and the purified water is recirculated into the aquaculture tank. It is this unique balance that leads to healthy animals and a productive crop.

Nitrifying microbes: engine of aquaponics

Bacteria are vital in aquaponics in that they metabolize the waste. Three critical types of bacteria exist to sustain the system: heterotrophic bacteria and two types of chemoautotrophic bacteria (or nitrifying bacteria). Nitrifying and heterotrophic bacteria are aerobic, making oxygen important to their health as it is to the fish and the plants. Organic nitrogen (solid fish waste) is decomposed to ammonia (NH_3) or ammonium (NH_4^+) by various heterotrophic microbes in the wastewater. This process of ammonification simultaneously fuels some of the bacteria's metabolic processes. Nitrifying bacteria change ammonia to nitrite, and nitrite to nitrate. This process of nitrification is carried out mainly by aerobic chemolithotrophic bacteria and consists of oxidation of ammonia to nitrite by ammonia-oxidizing bacteria (AOB, primarily *Nitrosomonas*), followed by oxidation of nitrite to nitrate by nitrite-oxidizing bacteria

(NOB, primarily *Nitrospira*). The overall reaction of nitrification can be written as (Haug and McCarty, 1972):

Nitrosomonas: $55\ NH_4^+ + 5\ CO_2 + 76\ O_2 \rightarrow C_5\ H_7NO_2 + 54\ NO_2^- + 52\ H_2O + 109\ H^+$

Nitrobacter: $400\ NO_2^- + 5\ CO_2 + NH_4^+ + 195\ O_2 + 2\ H_2O \rightarrow C_5\ H_7NO_2 + 400\ NO_3^- + H^+$

This nitrogen transformation eliminates ammonia from the water. Nitrate is not toxic to fish except at very high levels (96-h LC50 > 1000mg/L NO3-N; Colt and Tchobanoglous, 1976) and is the primary source of nitrogen for plants in hydroponic systems (Hochmuth, 1991; Resh, 1998). The system of water purification seen in this process is utilized by aquaponic plants and removes pollutants like nitrogen compounds from aquaculture effluent.

Nitrogen Transformation: Nitrogen cycle

Nitrogen is a naturally occurring element that is essential for growth and reproduction in both plants and animals. In aquaponics the nitrogen cycle is the most important process because it converts toxic nitrogen compounds to nitrate. Fish feed is the major source of nitrogen in aquaponic systems, once added the nitrogen cycle begins. On average, fish retain about one third of nitrogen in the feed, while the rest is excreted as ammonia.

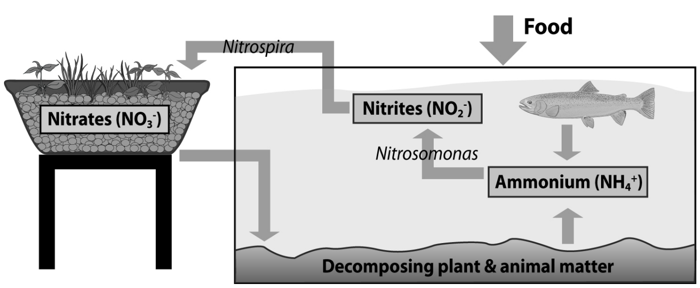


Figure 5: A diagram of nitrogen cycle in aquaponics.

In water, ammonia exists in two forms: un-ionized ammonia (NH_3) and ionized ammonium (NH_4^+). As one, the two forms are referred to as total ammonia nitrogen (TAN). Total ammonia is harmful to fish if allowed to accumulate in the system and is affected by the pH and temperature of the water. In general, less than 10% of TAN is in the toxic form (NH_3) when the pH is less than 8.0. This distribution increases dramatically as pH increases and warm water favors the toxic form (NH_3). Both unionized ammonia and nitrite can be harmful to fish at very low levels (Harmon, 2001; McGee and Cichra, 2000). Ammonium (the dominant ion under neutral and acidic conditions) and nitrite are oxidized according to the following stoichiometry (neglecting biomass growth):

Chemical process of Nitrification

Nitrosomonas: first step

$$NH4+ + 1.5O2 \rightarrow \; + NO2- + 2H+ + H2O + 84 \text{ kcal/mole of ammonia} \qquad (1)$$

Nitrospira: second step

$$NO2- + 0.5\ O2 \rightarrow NO3- + 17.8 \text{ kcal/mole of nitrite} \qquad (2)$$

Overall:

$$NH4+ + 2\ O2 \rightarrow NO3- + 2\ H+ + H2O + \text{energy} \qquad (3)$$

As shown in the above equation, the oxidation of every mole of ammonium produces 2 moles of acidity (first step of nitrification), which results in a pH decrease in poorly buffered environments. The nitrification process of bacteria naturally lowers the pH of an aquaponic system. Weak concentrations of nitric acid are also produced from the nitrification process as the bacteria liberate hydrogen ions during the conversion of ammonia to nitrate. Over time, the aquaponic system will gradually become more acidic primarily as a result of this bacterial activity as well as CO_2 respiration from fish.

Factors influencing nitrification: High nitrification at low pH

The main factors that affect nitrification rates are temperature, pH, and dissolved oxygen concentrations. The temperature for optimum growth of nitrifying bacteria is between 77-86 ºF (25-30ºC). Growth rate decreases by 50% when the temperature is decreased from the optimal to 64ºF (18ºC) and nitrifying activities cease when the temperature falls below 32ºF (0ºC) or above 120ºF (49ºC) (Goddek et al 2015). As with temperature, most living things have a very specific pH range in which they can survive, and an even narrower range in which they thrive.

The pH is one of the most important environmental parameters that can affect the activity of nitrifying bacteria. Autotrophic microbial nitrification is known to be highly sensitive to pH. A wide range of pH optima have been reported from research on the effect of pH on nitrification rate. The pH of approximately 7.8 produced the max growth rate of nitrifying bacteria for wastewater treatment processes (Antoniou et al., 1990). In aquaculture biofilters nitrification was reported to be most efficient from about 7.5 to 9.0 (Masser et al., 1999). Optimal conditions in aquaponics have been found to be within the range of 7 to 8.5 (Tokuyama, 2004). In a submerged biofilter investigation, a pH increase of one unit within a range of 5.0 to 9.0, produced a 13% increase in nitrification efficiency. (Villaverde, et al., 1997). In another study with four different biological filters (under gravel, fluidized bed, non-fluidized bed, and gravel bed) nitrification slowed significantly or stopped when pH dropped below 6.0 (Brunty, 1995). Generally, below pH values of 6.5, growth of autotrophic ammonia-oxidizing bacteria in liquid culture does not occur.

Table 3 Various pH optima for Nitrification

Environment	pH	Citation
Wastewater treatment plant	7.8	Antoniou et al., 1990
Aquaculture	7.5 - 9.0	Masser et al., 1999
Aquaponics	7.5 - 8.5	Tokuyama, 2004
Submerged biofilter	5.0 - 9.0	Villaverde, et al., 1997
Biological filters	Nitrification inhib <6	Brunty, 1995
Liquid culture	Nitrification inhib <6.5	Khan, et al 2014
Acid soil	3.3	Tyson, 2008

The causes of varying pH optima may be attributed to differences in substrate, effluent, or species of nitrifying bacteria present in the system. Inhibition of nitrification in acidic conditions has been attributed mainly to the exponential decrease in free ammonia (NH_3) with decreasing pH ($NH_3 + H+ \leftrightarrow NH_4^+$; pKa = 9.25) (Tyson, 2008). Free ammonia is considered to be the substrate for the primary enzyme ammonia monooxygenase, and the transport of free ammonia into the cells (unlike ammonium ions) is by passive diffusion. However, low rates of nitrification and the presence of autotrophic nitrifying bacteria in acid soils have been reported by researchers (Tyson, 2008) with a pH as low as 3.3. Since nitrification is reduced under low pH, a base is needed to buffer the acid produced during nitrification. Recommended pH ranges for hydroponic systems are between 5.5 and 6.5 (Hochmuth, 1991) and for aquaculture systems are btw 6.5 and 8.5 (Timmons et al., 2002). The pH of the water has a major impact on all aspects of aquaponics, especially the plants and bacteria. For plants, the pH controls the plants' access to micro- and macronutrients. At a pH of 6.0–6.5, all of the nutrients are readily available, but outside of this range the nutrients become difficult for plants to access. In fact, a pH of 7.5 can lead to nutrient deficiencies of iron, phosphorus and manganese (Goddek et al 2015). There are many biological and chemical processes that take place in an aquaponics system that affect the pH of the water, some more significantly than others, including: the nitrification process; fish stocking density; and phytoplankton.

Nitrifying bacteria experience difficulty below a pH of 6, and the bacteria's capacity to convert ammonia into nitrate reduces in acidic, low pH conditions. This can lead to reduced bio-filtration, and as a result the bacteria decrease the conversion of ammonia to nitrate, and ammonia levels can begin to increase, leading to an unbalanced system stressful to the other organisms. Fish have specific tolerance ranges for pH as well, but most fish used in aquaponics have a pH tolerance range of 6.0–8.5. However, the pH affects the toxicity of ammonia to fish, with higher pH leading to higher toxicity. Nitrifying bacteria function adequately through a pH range of 6–8.5 (FAO, 2012). Generally, these bacteria work better at higher pH, with the *Nitrosomonas* group preferring a pH of 7.2–7.8, and the *Nitrobacter* group preferring a pH of 7.2–8.2 (FAO). However, the target pH for aquaponics is 6–7, which is a compromise between all of the organisms within this ecosystem. Nitrifying bacteria function adequately within this range. In conclusion, the ideal aquaponic water is slightly acidic, with an optimum pH range of 6–7. This range will keep the bacteria functioning at a high capacity, while allowing the plants full access to all the essential micro- and macronutrients.

Nitrous oxide (N_2O) emission from aquaponics

Nitrogen is an element that can combine with itself or with other elements to make different compounds. It makes up about 80% of the Earth's atmosphere, while oxygen gas (O_2) makes up a little less than 20% of the atmosphere. In 2013, nitrous oxide (N_2O) accounted for about 5% of all U.S. greenhouse gas emissions from human activities (EPA, 2015). Nitrous oxide is naturally present in the atmosphere as part of the Earth's nitrogen cycle, and has a variety of natural sources. Nitrogen transformations in aquaponics can produce nitrous oxide through nitrification and denitrification. According to a study that determined N_2O emissions from aquaponics, nitrogen within the system could be converted to nitrous oxide depending on temperature, DO, ammonium and nitrate concentrations (Khanal 2016). Aquaponics might be an important source of N_2O with conversion ratio varying from 1.5 to 8.0% (Khanal 2016). Also denitrification is an anaerobic process in which heterotrophic bacterial species can take nitrate and reduce it back to nitrogen gas, which is released into the atmosphere. Therefore even with the benefits of aquaponics on the environment it still can have a negative impact by supplying excess nitrogen into the atmosphere.

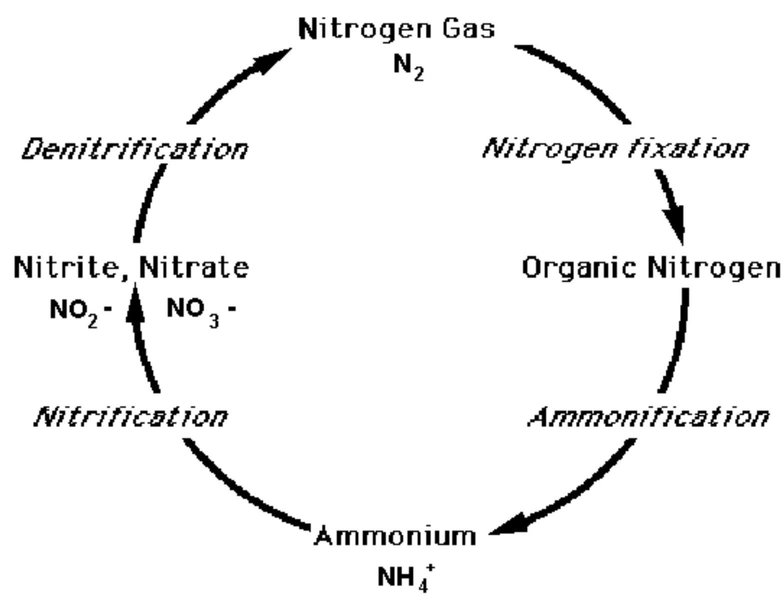


Figure 6: Simplified Nitrogen Cycle, italics denote processes and bold the different forms of nitrogen. In summary, nitrogen cycles through the air, water and soils, with many transformations mediated by the actions of specialized bacteria.

Some of these transformations require aerobic conditions while others occur only under anaerobic conditions. The best wastewater disposal systems take advantage of the metabolic needs of these bacteria to reduce the amount of nitrogen in the effluent.

Method and Materials

Water quality parameters

Water is the essence of aquaponics. The fish and the plants are entirely dependent on water for their survival; therefore monitoring the water quality is imperative to sustain a healthy environment. Dissolved oxygen, electrical conductivity, pH, temperature, and nitrogenous compounds are important parameters monitored for the health of the system.

Dissolved oxygen (DO) is the amount of oxygen dissolved in the water. Adequate DO is needed for the fish survival, bacteria (nitrification), and plant growth (respiration for roots). A YSI multi-parameter DO meter was used to monitor the oxygen (maintained at 5-mg/L) and temperature in the water. DO was achieved with the use of a 1-horse power blower connected to diffusers within the fish tank. Cold-water holds more DO than warm water, so proper aeration becomes more important as temperature rises. Tilapia can survive low DO levels, but supplemental DO should be added to support necessary microbial activity and plant root respiration.

Water temperature affects all aspects of aquaponic systems. Overall, a general compromise range is 18-30'C. Temperature has an effect on DO as well as on the toxicity (ionization) of ammonia; high temperatures have less DO and more unionized (toxic) ammonia. Also high temperatures can restrict the absorption of calcium in plants. Warm water fish (tilapia) and nitrifying bacteria thrive in higher warm temperatures of 22-29'C (FAO). Due to the year-round tropical climate, temperature typically is not a problem in Hawaii but varies depending on location. In Manoa temperature in aquaponics ranges from 22.0 – 27.0 'C.

Electrical conductivity (EC)

Conductivity is most important for plant production. EC measures the ability to conduct an electrical current. It is a direct measure of all ion content in the water (high ion content, high conductivity). The EC meter is temperature sensitive (standardized to 25'C) and is measured in micro/milli-Siemens/centimeter or ppm (uS/cm). EC was recorded using an electronic meter from aquatic ecosystems.

pH is an essential water chemistry property to measure because it affects the fish, plants, and the bacteria. Pinpoint pH monitor (single parameter) from aquatic ecosystems was used to target the pH between 6 and 7. A pH outside of this range can cause the plants to experience nutrient lock, in which the plant is unable to absorb readily available nutrients (Goddek et al 2015). This occurs according to the rules of acid/base chemistry and how plant roots absorb charged particles. Fish prefer a neutral pH, but accept pH levels between 6 and 8. Nitrifying bacteria work more quickly at basic pH 7.5-8.5.

Aquaponic Design

In this study, aquaponic systems were constructed and located outdoors at Hale Tuahine aquaculture facility at the Magoon Research Station, University of Hawaii at Manoa. A total of twenty-four individual, identical aquaponics systems were setup 2x12 in parallel. Recirculating trickling biological filtration system (biofilter) was used containing cinder rock medium from Big Island, HI. Each system consisted of: 100-gallon plastic fish tanks stocked with a density of 0.5-kg *Oreochromis niloticus*. The tilapias were obtained from Windward Community College (Honolulu, HI, USA). A blower was used to provide oxygen for fish growth by aerating the tank water and the tank was partially covered by the grow beds to prevent algal growth.

Figure 7 & 8: Aquaponics at Hale Tuahine. For the hydroponic component, trickle drainage was used in which the grow bed is continuously filled with the water coming up from the fish tank. Systems are under a tent to prevent acidic rainfall from contaminating the water.

System Design: ratio of plants to fish

A trickle system was used for water circulation. Along with cinder or lava rocks as media for hydroponic grow bed to perform filtering functions: mechanical (solids removal), mineralization (solids breakdown and return to the water), and bio-filtration. The solid support medium serves the dual purposes of providing structure for plant roots to grow in and surface area allowing proliferation of aerobic nitrifying bacteria, which are essential for converting nitrogen in the effluent to forms suited to the plants' nutrient uptake (Fox, 2012). The industry standard is at least 12-inches (300-cm) deep to allow for growing the widest variety of plants to provide complete filtration.

Nutrient Flux Hypothesis: Volume relationship

There must be a balance between the amount of fish waste and the ability of the bio-filter and the plants to convert that waste into plant food. Too much waste can overwhelm the bio-filter and leads to anaerobic conditions in which the fish suffer. Too little waste and there is not enough nutrients for plant growth. Therefore the initial fish stocking density was 1-pound of fish per 5-7 gallons of tank water. Fish fed as much as they will eat in five minutes, twice per day. An adult fish will eat approximately one percent of its body weight per day; while fingerlings will eat as much as seven percent. Since fingerlings were used an initial density of 0.5-kg was used because the fish growth will increase rapidly.

System startup cycle: bleached

Instead of cycling, the systems were bleached with regular bleach (Clorox brand) to clean the systems before use. Once systems were completely flushed (2 weeks) the 0.5-kg of tilapia fingerlings were stocked into each fish tank. The purpose of the bleach was to characterize the buildup of bacteria and nutrients within the water. When introducing fish into the system: system is fully cycled.

Tilapias have specific oxygen requirements that stem from their native environments. Because some tilapia come from African lakes they often suffer from poor water quality and have evolved to be extremely tolerant of relatively low oxygen levels.

Tilapia are a common choice of fish in aquaponic systems primarily because tilapia tolerate poor environmental conditions. Equally important is the fact that there is a market for tilapia, which are prized for their white, mild, and flakey flesh. Tilapia is the fish used exclusively in aquaponic operations in Hawaii. Tilapia tolerate low DO levels (e.g., 0.2 ppm); tolerate high total nitrate levels (>400 ppm); tolerate high total ammonia nitrogen levels (e.g., >90 ppm) at pH 6.0; and tolerate low pH levels (<5.0) (Goddek et al 2015). Tilapia is the most commonly cultured fish species in aquaponic, because of their high tolerance to fluctuation of oxygen, ammonia and dissolved solids.

Plumbing

The circulatory system for an aquaponic ecosystem consists of the plumbing components. Elements include blower, pvc pipes for water and airlifts, and water flow rate. The blower moves air up the pipe along with pockets of water from the fish tank. Trickle system was used in which water enters the grow bed and trickles to the bottom, returning back into the fish tank through the media blocker (perforated pipe to block the media while allowing water to flow freely.

Operation: seed germination, transplant, harvest.

Plants are started for aquaponics the same way they are for a soil garden – by seed, cuttings or transplant. Seedlings are grown in ½ perlite and ½ potting soil media. Two weeks after germination, the seedlings are transplanted into the grow bed. Chili peppers take 2 months and lettuce takes 2 weeks to grow until they are transplanted. Seedlings were watered thrice daily: early morning, afternoon and evening. Harvest according to ctahr.hawaii.edu/oc/freepubs on-farm food safety.

Maintenance

General Maintenance: Both fish and plants growing in their respective systems need regular visual and technical monitoring. If adjustments need to be made, they need to be made immediately. Soil acts as a buffer to plants when deficiencies occur. In aquaponics, both the plants and their roots are in direct contact with the water solution and react fast in a negative manner to any deficiencies or imbalances.

Daily: Fingerlings are fed with commercial fish feed twice a day and are visibly evaluated by checking swimming and feeding behaviors of fish in all systems. Fish are fed to satiation by increments (i.e., feed a little; if fish are eating well, add more). This will avoid food waste as well as poor water quality. Ten minutes after feeding, the tanks are checked for excess feed leftover.

Weekly: Water levels are checked and adjusted accordingly. It is important to note that source water includes chlorine therefore additions must be made slowly (5 gallons/hr). Check for algae growth in siphon area. Clean if necessary.

The pH of tank water and the water quality parameters are measured every week to monitor the accumulation of ammonia and nitrite. Weekly water chemistry includes: temperature, pH, DO, total ammonia nitrogen, nitrite and nitrate – are taken from each system.

Results

Water quality: nitrogenous compounds

For the preliminary trial the fish tanks were initially bleached with Clorox to ensure no bacteria colonies were present at the start of the experiment. Figures 8 & 9 demonstrate the buildup of nitrogenous compounds within an aquaponic system for six months. Since there are no bacteria colonies present within these systems bacteria growth was not established until after the first two months of cycling. The gradual accumulation of ammonia within the first two months shows that the bacteria have not colonized to a sufficient amount. Once the bacteria population reaches a certain threshold then the ammonia and nitrite is metabolized and concentrations level off within the system (July). Nitrite concentration remained low throughout demonstrating that the *Nitrospira* established first. Nitrate and ammonium are the most common forms of nitrogen taken up by vegetable crops (Cocks and Simonne, 2003). The optimum nitrate to ammonium ratio for vegetables grown in hydroponics is 74:25 (Tyson, 2004).

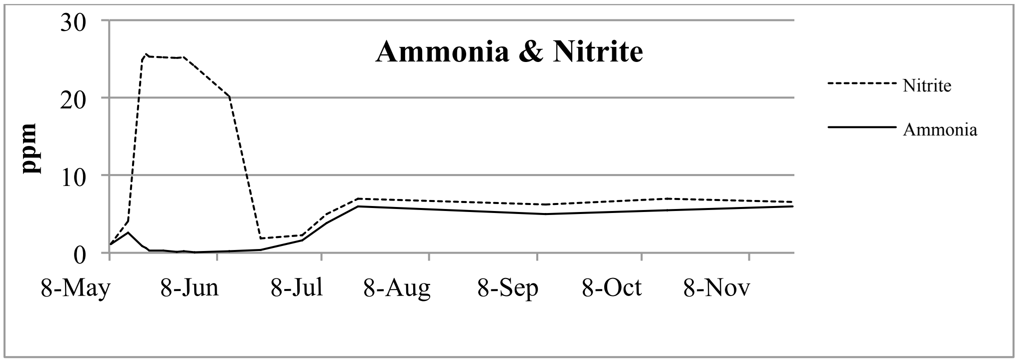


Figure 9 Bleached water quality: Ammonia and nitrite.

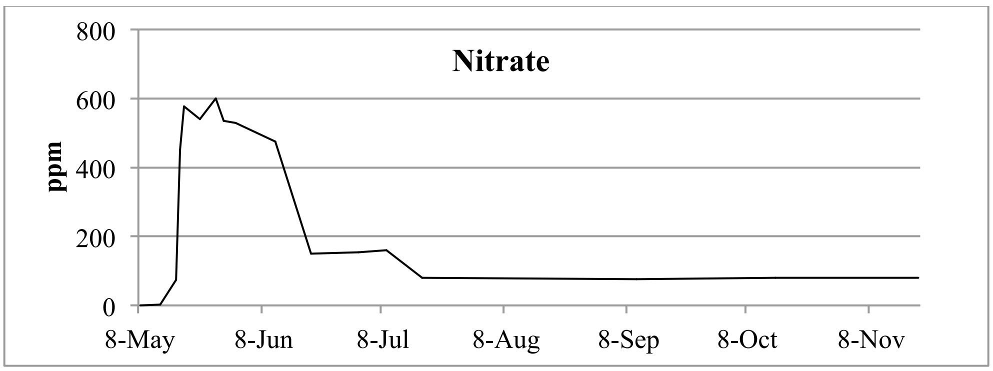


Figure 10 Bleached water quality: nitrate.

Water quality: pH

Temporal Change in pH Without Remediation

May 2013 – March 2014

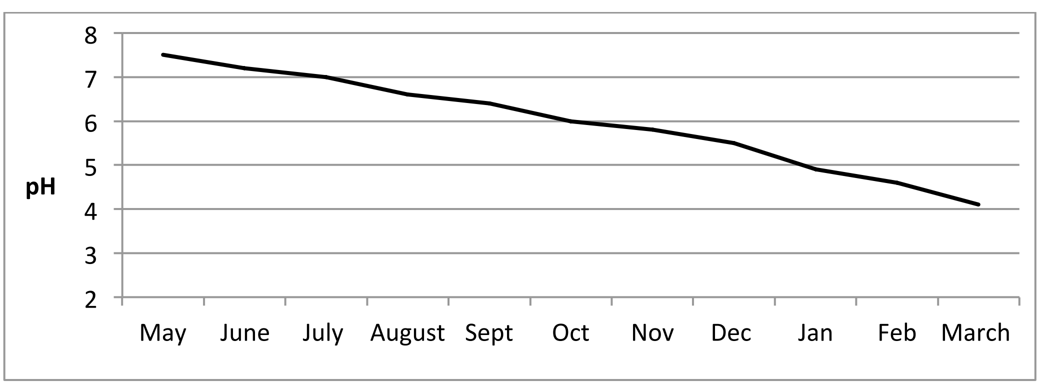


Figure 11: Gradual decrease in pH in one year.

One of the most complex and important subsystems of recirculating aquaculture is the bio-filtration and removal of fish waste. Recirculating systems must incorporate both solids removal and biological filtration into the water reconditioning process to achieve proper WQ for fish and plants (Harmon, 2001). This is done through the process of nitrification where nitrogen source from the fish is metabolized into nitrate by the bacteria. The integration of hydroponics with aquaculture is believed to be able to improve the water quality. However over prolonged period of time, pH of the water becomes acidic due to accumulation of hydrogen ions from nitrification, resulting in a toxic acidic environment. The fish, the plants, and the nitrifying bacteria rely on the same recirculating water for optimum growth hence water quality parameters have to be favorable for all three organisms in a self-sustaining aquaponic system.

Table: Fish density distribution

	Density	Length
Initial	**0.5-kg**	**5-cm**
Final	**3.0-kg**	**16-cm**
Growth	**2.5-kg**	**11-cm**

Condition factor (K) is based on the weight-length relationship; it is the measure of fatness or plumpness of aquatic organisms. K was calculated to be 0.19%. Feed concersion ration (FCR) is the mass of food eaten divded by the output over a specific period of time (FCR for tilapia is normally 1.5). FCR was calculated to be 0.65. This FCR is low particulary for fingerlings (when relative growth is large) and increases for older animals (when relative growth tends to level out). The tilapia fingerlings started with 0.5-kg. At the end of the first trial the fingerlings grew six times the original weight and tripled in length over a six month period. The distribution of weight and K of each fish within the experiment are shown in the figures below.

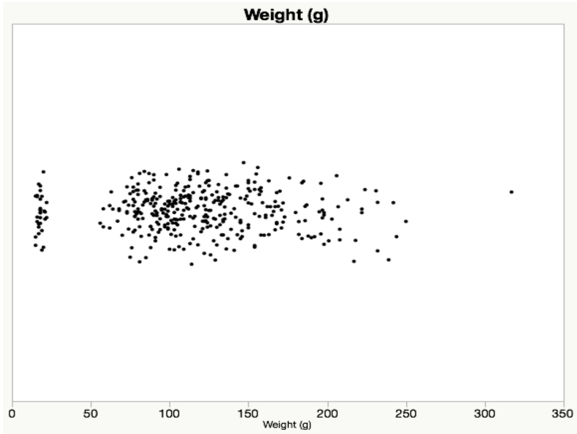


Figure 12 Distribution of fish weight (g).

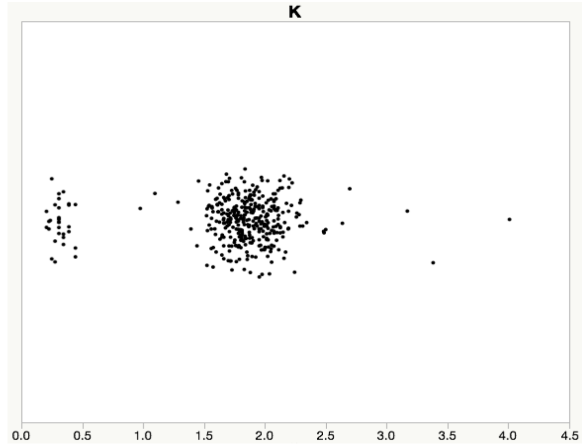


Figure 13 Distribution of condition factor.

Table 4 Super Chili Pepper yield using fresh weight (g) for 10-day harvest.

Day	**Yield (grams)**
1	521.4±12.29
2	43.45±1.58
3	92.01±5.61
4	132.7±8.79
5	-------
6	-------
7	262.4±8.87
8	201.6±6.75
9	425.8±10.63
10	93.83±4.32
Total	1773.3 ±171.7

Chapter 4

Capsaicinoid Analysis in Aquaponics

Introduction

Capsicum Background

The genus Capsicum, which originates from tropical and humid regions of Central and Southern America, belongs to the *Solanaceae* family and includes peppers of important economic value. It is an annual or perennial shrub with fruits of various size, shape, flavor, color and pungency. There are thought to be 25-30 species of Capsicum, three of which are extensively cultivated and have a hot and pungent berry: *Capsicum annuum, Capsicum frutescens, and Capsicum chinense*. Capsicum fruit has been used worldwide as chili peppers and is one of the oldest and most popular spice in the world. The ripe fruits of the different varieties of peppers have also been traditionally used as natural food colorants. Chili plants were first cultivated between 5200 and 3400 BC by the native Americans and are among the oldest cultivated crops (Meghvansi et al. 2010). World production of hot peppers is mostly in tropical countries, e.g., India, Indonesia, Myanmar, Bangladesh, Pakistan, and Thailand (FAOSTAT, 2009). Red pepper or capsicum is consumed worldwide with an always-increasing demand, in the fresh form and as processed natural colorants, in the form of paste, paprika, and oleoresin. Its popularity derives from a combination of different factors such as color, taste and pungency. Capsicum is an important agricultural crop, not only because of its economic importance, but also due to the nutritional and medicinal value of its fruits.

Chili Spice in food, medicine, and pharmacology

Chili is an indispensable spice used as a basic ingredient in a variety of cuisines all over the world. As a general rule, spices stimulate the appetite, enhance liver function, and increase blood circulation. For example, turmeric is a noted antiseptic and is used to treat skin diseases; coriander with ginger made into a tea acts as a decongestant (Burst of

Flavor); Fragrant spices are said to be able to calm the nerves and soothe the senses, not just please the palate. Chilies are known to aide indigestion and are employed whole or ground and alone or in combination with other flavoring agents, primarily in pickles, stewed or barbequed (Ravishankar et al., 2003). The colors exhibited in Capsicum are due to a mixture of esters of capsanthin, capsorubin, zeaxanthine, crytoxanthine and other carotenoids (Kothari et al., 2010). These extractable colors of chili pepper fruits are used extensively in the food processing industry, such as meat products (sausages), cheeses, butters, salad dressings, condiment mixtures, gelatin desserts and other processed foods (Govindarajan, 1986). The continuing interest in nutrition and the dynamic influence of ethnic and international cuisines has sparked a keen interest in spices.

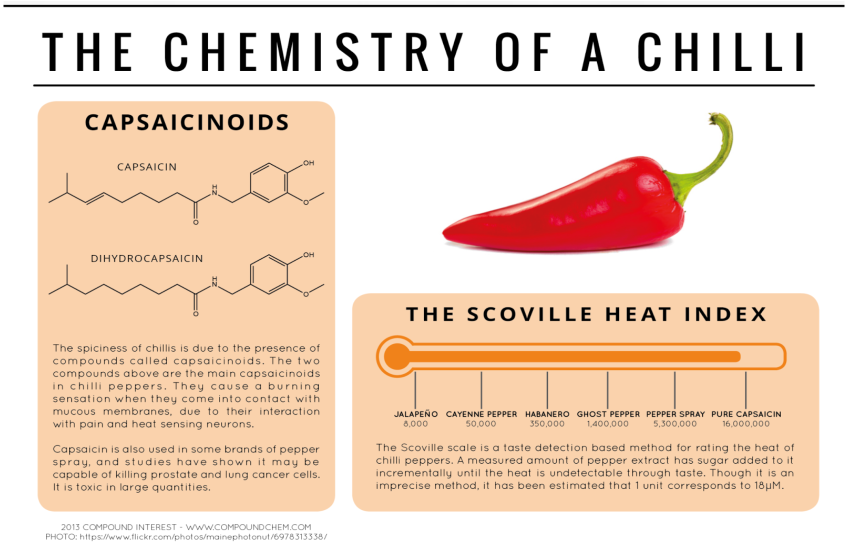
Interest in chili compounds extends far beyond their roles as flavor ingredients in food; they also have nutritional and therapeutic implications. Studies have revealed that chili is a highly nutritive fruit (Tripathi & Mishra 2009; Ismail et al. 2011) with an excellent source of vitamins C (ascorbic acid), A, B-complex and E along with minerals like folate, potassium and thiamine. Beta-carotenoids, and vitamins C and A in chilies are powerful antioxidants that destroy free radicals (Simmone et al., 1997). Despite the nutritional importance of chilies, the folklore of its medicinal relevance is also well established. For centuries chilies were utilized as medicine in Ayurveda preparations as an oil extract and is one of the major ingredients in Mayan therapeutic remedies. In Western medicine, it is used as a rubefacient in the form of Capsicum tincture and is listed as an official drug in several pharmacopoeias (Thapa, 2009). Currently capsaicin is used in the form of non-prescription (in the United States) or prescription (in the European Union) topical analgesia. It is also used as a high-dose dermal patch, to relieve the pain of peripheral neuropathy such as post-herpetic neuralgia caused by shingles. Capsaicin also provides relief in arthritis and respiratory ailments (Mazzone and Geraghty 1999). Moreover, its continuous demand and wide application in the industry makes Capsicum fruit an important ingredient in the food and medical industry.

Due to dietary and nutritional significance, chilies display pharmacological importance in human health. A number of properties, such as antioxidant, antimicrobial, antiinflammatory, cardio-protective, anti-carcinogenic, have been extensively studied in recent years (Khan 2014). Capsicum fruit is of ethnopharmacological importance in that it is used as a circulatory stimulant, which has been traditionally, used in most cuisines and food products due to its distinctive flavor, color and aroma. Capsaicin has attributed pharmacological effects since ancient times. The plants have been used as folk remedies for dropsy, colic, diarrhea, asthma, arthritis, muscle cramps and toothache (Ravishankar et al., 2003). Not until the past 20 years has extensive research been done to determine specific applications, including the gastrointestinal tract, for weight-loss and as an analgesic. These finding have helped to support further research into therapeutic properties attributed to the capsaicinoids. Many biological effects and important pharmaceutical properties have been recognized to capsaicinoids that possess physiological, pharmacological and antimicrobial activities, used in the treatment of several painful and inflammatory conditions. Capsaicin, the health promoting phytochemical in Capsicum fruit, is widely used in food, medicine and pharmaceutical industries.

Capsaicinoids

One of the main characteristics of chili pepper fruit is its spicy taste due to the presence of a family of compounds known as capsaicinoids. Of these compounds, capsaicin and dihydrocapsaicin account for approximately 90% of capsaicinoids in chili pepper fruit and usually their amount is determined for pungency characterization. Pungency, the organoleptic sensation of heat, is a major quality-determining factor in Capsicum and is attributed to capsaicinoids (Barbero, 2006). Capsaiciniods are all amides formed from enzymatic condensation of vanillylamine and fatty acids of different chain lengths. The structural differences among capsaicinoids depend on the number of lateral chain carbons (R) and the presence or absence of unsaturations. Capsaicin and dihydrocapsaicin are the two most potent capsaicinoids and their molecules differ only in the saturation of the acyl group. Capsaicinoids are synthesized in the interlocular septum and consequently the portion of the fruit richer in capsaicinoids is the placenta tissue (Prasad et al., 2006). The whitish flesh of the placenta that bears the seeds is the hottest part of the fruit, containing a much greater concentration of capsaicin than does the fruit wall. The structures for capsaicin compounds that elicit a response in the cell are divided into three regions: aromatic ring, amide bond, and hydrophobic side chain. It is known that a hydrophobic group, (such as an octyl chain or substituted benzyl or group) is required for high potency (Escogido, 2011).

Figure 14: Capsaicin is a crystalline, lipophilic, colorless and odorless alkaloid.



Although various methods have been reported to determine the capsaicin content studies suggested that capsaicin content varies considerably in Capsicum fruits (Appendino, 2008). The content of phytochemicals in plants is generally influenced by several factors such as genotype (variety or hybrid) and environmental conditions such as agro-climatic conditions and cultivation technique (Giuffrida, 2013). Levels of capsaicinoids can also vary with stage of development (maturity) at harvest, storage and processing conditions, the size of the fruit, and even the location of the fruit on the plant. Capsaicinoid accumulation is regulated by a genetic and an environment interaction (Zewdie and Bosland, 2000). In summary, while capsicums all have phytochemicals and antioxidant nutrients, the types and the amounts they have differ within varieties and harvesting stage.

Plant secondary metabolites, such as capsaicin, are unique resources for pharmaceuticals, food additives, flavors, and industrially important biochemical. Accumulation of capsaicinoids often occurs in chili plants subjected to stress including environmental factors such as the supply of water, minerals and carbon dioxide (Ramakrishna, 2011). A wide range of environmental stresses (drought, alkalinity, salinity) is potentially harmful to plants, causing adverse effects on the growth and productivity of crops. However, elicitation has been widely used to increase production of secondary metabolites that allow plants to adapt to the environment and overcome these stress conditions. In chilies, capsaicinoid production is stimulated and used for plant defense against herbivores and pathogens. Influence of stress increases capsaicin because growth is often inhibited more than photosynthesis, and the carbon fixed is predominantly allocated to secondary metabolites (Ramakrishna, 2011). These plants are manipulated to produce these compounds, which are widely used industrially for food, medicine and pharmaceuticals. The irritant effect of the capsaicinoids, particularly dangerous for the mucosal tissues, is at the basis of another industrial application, which is the production of defensive pepper spray for riot control.

Chilies possess a range of important compounds such as capsaicin that are produced for plant defense against pests. However, in this study it was used as an indicator of stress on capsicum to see which treatment is suitable for the system. Therefore, the known capsaicin content is prerequisite for optimizing crop quality in aquaponics. Methods have been reported to determine the capsaicin content, however there are no data available on capsaicin content of Capsicum fruits in aquaponics. The goal of the current experiment is to quantify the distribution of capsaicinoids in hot pepper fruits as influenced by fertilizer treatment and maturation level. This could assist producers to decide the optimum harvest date to maintain the quality of produce and increase the health benefits of these compounds.

Method and Materials

Field Experiment: Plant Material and Growing Conditions.

A 180-day field trial was conducted at Hale Tuahine, Magoon Research Facility in Manoa Valley, HI in spring of 2015 to evaluate buffering capacity of treatments in aquaponics with *Capsicum frutescens* (Hawaiian chili pepper). Seeds were purchased from Agriculture Diagnostics at University of Hawaii at Manoa. Seeds were sown, in June 2014, in 50% perlite and 50% potting soil and transplanted into aquaponic systems eight weeks after sowing. After 90 days of treatment application, peppers were harvested weekly for one month measuring fresh weight and yield. Fruits were harvested at maturation with similar size and picked randomly from different parts of the plant to obtain a representative sample. Samples were stored in refrigerator for one week and transported to the laboratory for analysis. All samples were freeze-dried with liquid nitrogen for ten minutes and placed in vacuum dryer for forty-eight hours. Peppers were cut in half to facilitate the drying process. The dried fruits were homogenized with seeds using a mortar and pestle and stored in the freezer until further analysis.

Experimental Design and Treatments

Twenty-four identical systems were used in a completely randomized design using six fertilizer treatments with four replicates. Harvest (90-120 days after transplanting) occurred for one month from each experimental unit (aquaponic system). Each system contained a biomass of 1.5-kg tilapia species (*Oreochromis aureus* and *Oreochromis hornorum*) grown in 400-L freshwater tanks associated with two ebb-and-flow 25-L bio-filters (cinder rocks). Tilapia were fed 2.5-grams of commercial trout feed daily. The Capsicum fruits were grown with different fertilizer treatments and market mature fruits were analyzed for capsaicin content using rp-HPLC.

Treatments for the experiment were used to assess buffering capacity or alkalinity, which refers to water's ability to keep the pH stable and is highly dependent on the amount of minerals dissolved in the water. Treatments were tested on Hawaiian chili peppers cultivated under acidic pH conditions. The goal of the treatment is to remediate pH for the aquaponic system to function efficiently in a symbiotic environment. The treatments include: 1.) potassium carbonate K_2CO_3, 2.) calcium carbonate $CaCO_3$ in the form of oyster shells, 3.) potassium nitrate KNO_3, 4.) calcium nitrate $Ca(NO_3)_2$, 5.) potassium carbonate + oyster shells, and 6.) control. Two levels of KCO_3 and two levels of oyster shells are used (0 and the amount of buffer needed to remediate pH). For positive controls KNO_3 and $Ca(NO_3)_2$, are used; they have no pH effect, only nutrient or fertilizer effect. The amount of potassium or calcium used in KCO_3 and oyster shells treatments is used to calculate how much nitrate is needed for fertilizer treatments (according to amount of K or Ca in carbonate treatments).

Treatments are buffers and are used for their capacity to remediate pH under acidic conditions in a recirculating aquaponic system. In order to understand the treatment design, the distinction between the physical attributes of a base and a buffer must be understood. For example the powder form of KCO_3 in solution is a base and has an immediate effect on pH once applied. Once the base is used up the pH returns back to acidic conditions within 48 hours. This fluctuation causes stress to the fish and plants inhibiting their growth. On the other hand, 2-mm oyster shells are considered a buffer due to its surface area, causing a consistent increase in pH towards neutrality (allowing the system to slowly absorb the buffering component). As a result of the surface area of the particular treatment the pH will be remediated for varying lengths (days to months). Thus treatment schedule is designed so that the pH will be stable and neutral for the duration of the experiment. Since the powder form of the base KCO_3 remediates the pH temporarily and returns to original acidic pH after 48-hours, the treatment will be applied thrice weekly along with the nitrate treatments in order to compensate for the fertilizer effect. Oyster shell treatment is applied daily (sits in a mesh bag inside fish tank). Thus, the experiment is designed to lead to recommendations over a wide range of conditions.

Determination of Capsaicinoid content.

Capsicum belongs to the *Solanaceae* plant family that produces secondary metabolites known as capsaicinoids for protection against phytopathogens. Growing interest in capsaicin has led to more sensitive and faster characterization techniques. Several methods are available for the identification and quantification of capsaicinoids, but HPLC is considered the most reliable and rapid method (Yao et al., 1994). Capsaicin is known to be a stress compound in peppers and in the experiment it is used as an indicator of plant health. Various treatments were evaluated for different components in the system including pH, crop yield and quality. To distinguish which treatment yields stable concentrations of capsaicin HPLC was used to quantify capsaicin in Hawaiian chili peppers cultivated in aquaponics.

Sample Preparation

Three fresh peppers were sampled from each aquaponic system and pooled according to their designated treatment. Samples were stored in refrigerator each week until freeze-dried in laboratory. The caps were snapped off and discarded and peppers were sliced horizontally to ease the drying process. First the peppers were freeze-dried with liquid nitrogen for 10 minutes and then samples were transferred into a vacuum dryer set at -80℃ for 48 hours. The moisture content was determined by weighing the sample before and after the drying process. Dried samples were then ground by hand in a mortar and pestle and stored in the freezer until further analysis.

Figure 15 Red and green dried chili pepper samples ground using mortar and pestle.

Extraction of Capsaicinoids.

Capsaicin was extracted, separated, and quantified using HPLC following the ‘long run” method according to Collins et al. (1995). A 5:1 ratio of dried chili powder (mg) to methanol (mL) was placed in 2-mL plastic vials. A 5.0±0.5-mg quantity of chili powder was used, depending on the amount of sample available for processing. Vials were capped and placed in an 80℃ water bath for 4 hours and swirled manually every hour. Samples were removed from the water bath and cooled to room temperature. All samples were centrifuged for 5 minutes at 5000 rpms and 1mL of supernatant was transferred into a new vial. The colors of the extracts corresponded to the surface colors of the peppers. Samples were stored in the freezer until analyzed.

Apparatus and Chromatographic conditions.

The liquid chromatography system used was a Waters-2690 separations module interfaced with a Waters 996 photodiode array detector (PDA). Data acquisition and analysis was achieved with Waters Millenium^{32} Version 3.20 software (Bedford, MA, USA). The reverse-phase chromatographic column was a Phenomenex, Kinetex C-18, 150x4.6 mm. Detection was set at 280 nm. HPLC operating conditions to determine total heat units included ambient temperature (27°C), a flow rate of 1 mL/min, and a run duration of 20 minutes. The mobile phase was isocratic, 60% 9:1:0.1 Triethylacetate (900 mL MeCN, 100 mL dH2O, 1 mL TEA, Solvent A) and 40% of 1% acetic acid (1 mL acetic acid in 1L 100% methanol, Solvent B). HPLC-grade reagents were used and all solvents were filtered and degassed using an all-glass filter holder. Mobile phase was used as diluent during the standard and test sample preparation. The capsaicinoid extract (60 uL) was diluted with 300-uL solvent mixture (280-uL of Solvent A and 20-uL of solvent B). Triplicate injections were run for each sample with a 10-uL aliquot for each injection.

Preparation of Standard Solutions.

Standards of *8-methyl-n-vanillyl-6-nonenamide* (capsaicin) and *8-methyl-n-vanillyl-nonanamide* (dihydrocapsaicin) were obtained from Sigma Chemical Co. (St. Louis) and were used for retention-time verification and instrument calibration. Standard solutions of 5 and 50-ppm were prepared in Solvent A by dilution of a 1000-ppm stock solution. 10, 20, 40, and 60-uL injections of each standard dilution were used for external multi-level calibration. Standards prepared by dilution were analyzed concurrently with all pepper extract samples to insure consistency in HPLC column behavior and certainty in measured signals. Each sample was subjected to triplicate injections for HPLC analysis.

Linearity

The linearity of an analytical method is its ability (within a given range) to obtain test results that are directly proportional to the concentration of an analyte in the sample. Linearity was determined by plotting the peak area of each standard against the concentration of the analyte. The correlation coefficient and slope of the calibration curves were calculated and reported. Quantification of capsaicinoids was calculated using the relative contribution of each capsaicinoid UV response peak to the sum of all the peaks contained in the capsaicin mixture. Concentrations were calculated relative to original dried pepper weights. Each sample was injected in triplicate replication and the mean of the HPLC runs was used for the data analysis. The raw HPLC data was transformed to parts per million (ppm).

Statistical Analysis

A one-way ANOVA analysis was conducted for each measurement using SAS 9.4. A multivariable analysis was used to see if there is any significant difference between the treatments and variables measured. The independent variables consisted of treatment, pH, and temperature. The dependent variables included pepper yield (fresh weight in grams) and fish density (grams). A multivariable analysis was used because there are more than two dependent variables being measured. From this a linear regression analysis was conducted to determine if there is any correlation between the treatment and yield or fish growth.

Additional Measurements and Assumptions

Aquaponic systems require monitoring of certain water quality parameters to operate effectively. Water quality parameters that were measured include: temperature (°C), dissolved oxygen (ppm), pH, electrical conductivity (EC), and nitrogenous compounds. Target pH is 6.0-7.0, which is a compromise between the optimal pH ranges of the fish, plants, and bacteria. pH monitored weekly using probe meter and then every two weeks once system is stable. Once treatment application starts, pH is monitored weekly. If data is missing in case of pest damage or some other environmental factor an analysis of covariance will be used to estimate the missing data. To estimate missing data the covariate used is the additional measurements taken such as fruit yield or weight and fish density. Thus weekly measurement of pH will be used as a covariate for capsaicin content in pepper analysis.

One assumption made in the experiment is that pH is affected only by nitrification and the addition of treatment buffers. Sources of environmental variation are controlled using various methods. First the entire 2x12 setup is under a canopy to keep acidic rainwater out of system water. Second each tank is covered half way to inhibit algae growth in tank water. Lastly, integrated pest management is used to attract beneficial organisms by incorporating nesting blocks and insectary crops such as buckwheat and sun hemp. Several procedures have been used to control environmental factors however in the analysis this variation can be minimized by measuring a covariate such as pH, initial plant height, or amount of sunlight or temperature. Some factors pertaining to weather or accidental leaks are inevitable.

To summarize, several treatments are being compared at different levels and the factor of interest is capsaicin content. The hypothesis to be tested is that there is a difference between treatments in capsaicin content and yield. Treatments are designed to measure specific effects including buffering capacity (remediate pH) and fertilizer effect (nutritional benefit), yielding in varying concentrations of capsaicin. The primary goal of the experiment is to determine which treatment yields stable concentrations of capsaicin in order to make a recommendation to aquaponic farmers in the community.

Results

Water Quality: pH

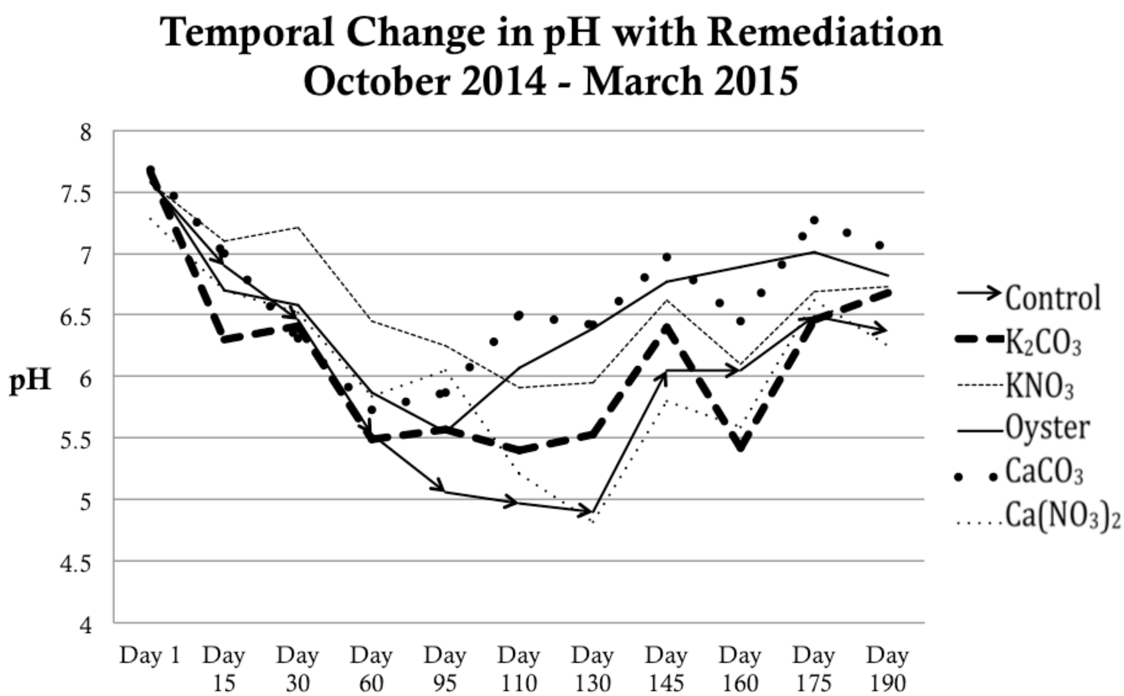


Figure 16 Temporal change in pH with remediation from November 2014 to April 2015. Treatment application began in February (Day 95). Fluctuation in pH caused by weekly water addition and surface area of buffer. General trend shows that pH for oyster shell is stabilizing towards neutral pH 7.

Once pH levels were below 5.5-treatment application started in February (Day 95). As a result of treatment application pH fluctuates for two predominant reasons: 1) due to weekly addition of water that must be added because of evaporation and 2) buffering capacity of chemical treatments can only maintain pH for a designated period of time. For example, the chemical treatments (potassium and calcium carbonates) remediates pH immediately but only for 48 hours. The surface area of the chemical (or powder) treatments allows it to be absorbed quickly causing a rapid change in pH. The controls (potassium and calcium nitrates and absolute control) had no pH effect, however pH fluctuation still occurred because of weekly water addition, especially during the summer months when temperature rises. Temperature of the environment during the day caused the water to evaporate (about 5% of the water is added weekly to compensate for evaporation). Unexpectedly potassium carbonate was not efficient at pH remediation since the potassium nitrate pH was higher throughout. For carbonate treatments calcium carbonate was more adequate than potassium carbonate. Ranking of treatments from efficient to inefficient buffer: oyster shell, calcium carbonate, potassium nitrate, potassium carbonate, control, calcium nitrate. Overall, oyster shell treatment maintained pH levels to neutral within the time frame of the experiment.

Water Quality: Nitrogenous compounds: total ammonia nitrogen, nitrite, and nitrate.

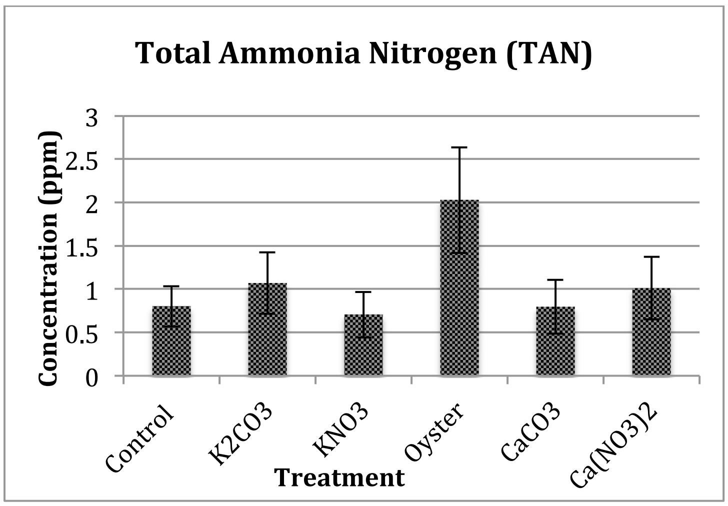


Figure 17 $p < 0.05$ for oyster treatment. TAN toxicity.

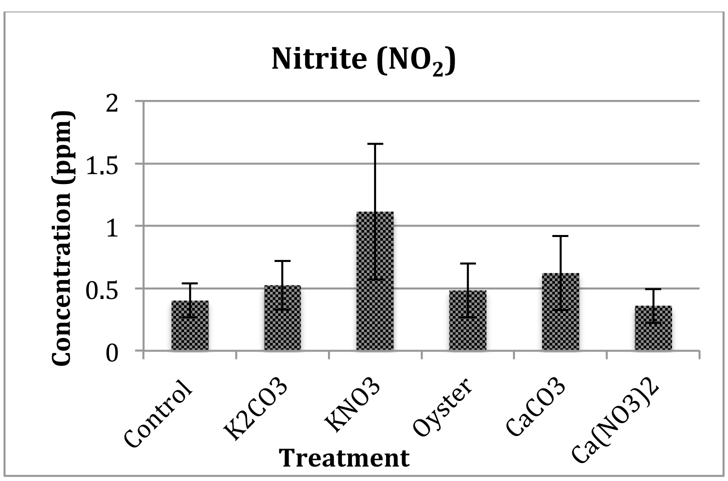


Figure 18 Nitrite toxicity for KNO_3. p value not significant ($p > 0.05$)

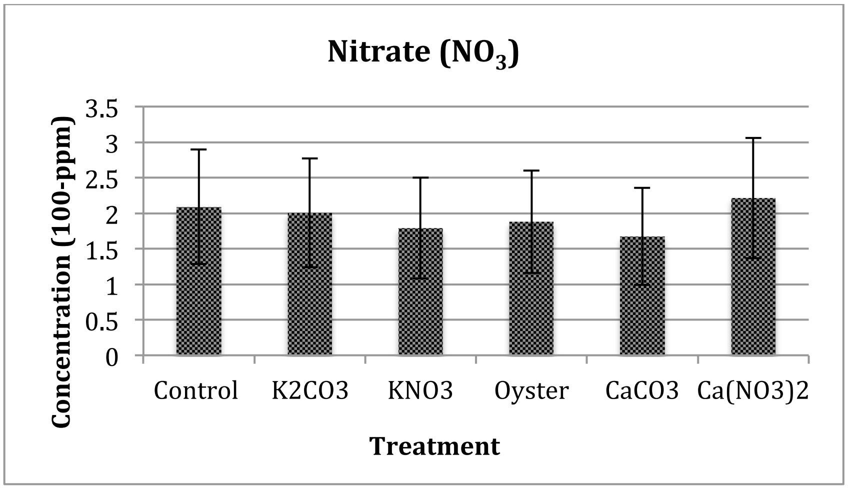


Figure 19 Nitrate distribution against treatment.

In the above Figures 17, 18, and 19 the distribution of TAN, NO_2, NO_3, respectively is plotted against each treatment. Oyster treatment contained very high concentration of ammonia (7.5-mgN/L). The high ammonia and low nitrite concentration in the oyster treatment shows that the *Nitrospira* bacteria are not present or there is insufficient amount of bacteria that cannot metabolize the ammonia in the system. Calcium carbonate treatment also contained high amounts of ammonia (3.5-mgN/L) as well as nitrites (3.0-mgN/L). The concentrations of ammonia and nitrite in the systems are enough to be toxic for fish. However no fish death occurred in these treatments; since tilapia can tolerate various environmental conditions they most likely adapt to their surroundings. Water quality of nitrogenous compounds for K_2CO_3, KNO_3, and $Ca(NO_3)_2$ were similar to that of the control. Therefore it is difficult to determine which treatment is adequate in cycling nitrogenous compounds for nitrification. Overall nitrates ranged from 1.5 to 2-100 mgN/L, which shows that nitrification is occurring even at low pH.

Fish Growth

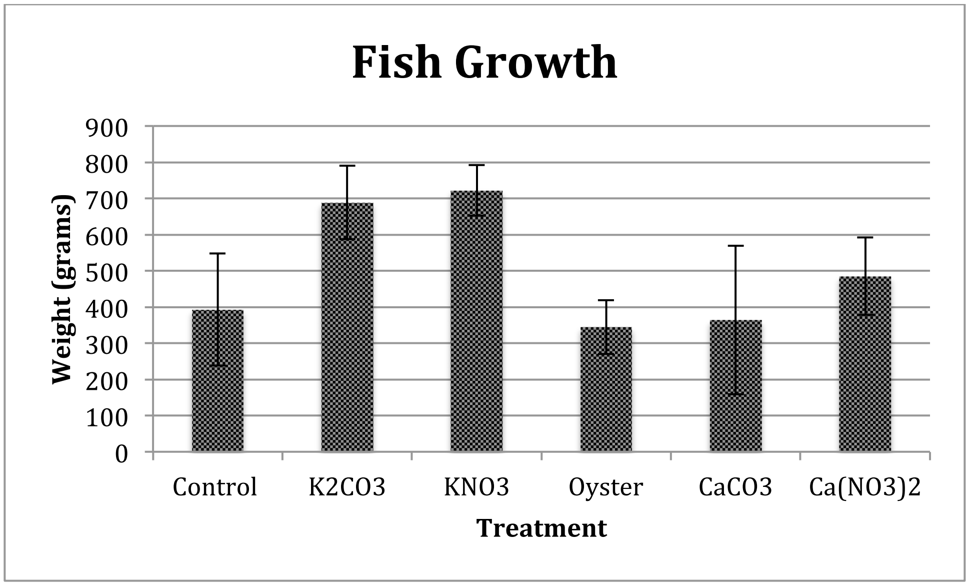


Figure 20 Fish density or growth in grams. Fish growth calculated as difference between the final and initial fish weight before and after the experiment. Nitrite toxicity observed for KNO_3 and TAN toxicity for oyster. $p > 0.05$ for oyster treatment demonstrates decline in fish growth is significant.

The fish were weighed and measured before and after the experiment. The graph demonstrates the growth of fish. Fish growth is consistent with water quality data in Figures 17 & 18. High ammonia and nitrite levels lowered the growth of the fish (oyster treatment $p > 0.05$). For example oyster and calcium carbonate treatment had the lowest density of fish overall, even lower than the control, due to ammonia and nitrite toxicity respectively. Calcium nitrate treatment, with high nitrates in the system, did not affect fish growth as expected. High nitrate did not affect fish growth in this study; however high ammonia and nitrite concentrations did have an impact on fish growth overall. For example oyster and Calcium carbonate had low fish growth compared to other treatments because of high concentrations of ammonia and nitrite in the system.

Hawaiian Chili Pepper Yield

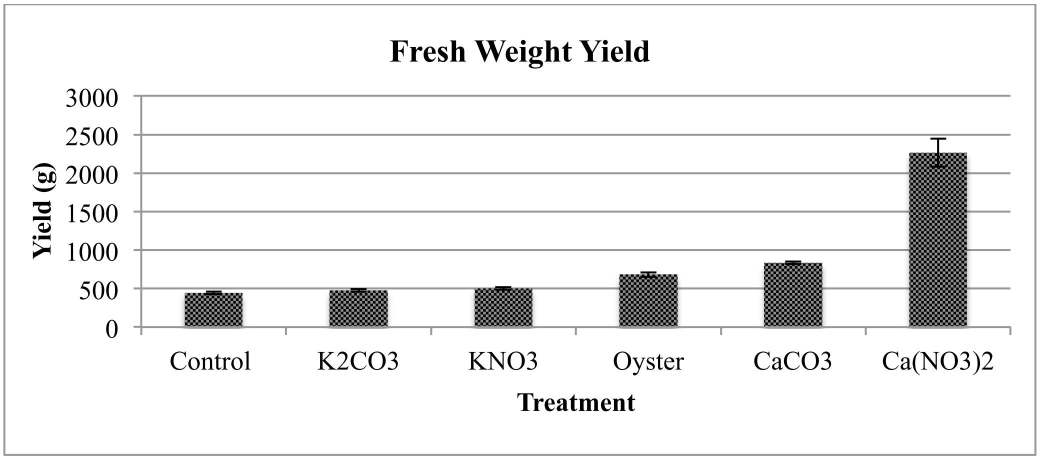


Figure 21 (above) Pepper yield (fresh weight in grams) raw data highly skewed due to pest damage; significant difference seen in $Ca(NO_3)_2$. Statistical analysis after log transformation: $p > 0.05$; CV = 27.12; $R^2 = 0.178$

Table 5 (below) Total pepper yield (raw data); data not normalized.

Treatment	Pepper Total Fresh Weight (Grams)
Control	446.49
K_2CO_3	473.55
KNO_3	500.61
Oyster	681.42
$CaCO_3$	835.17
$Ca(NO_3)_2$	2263.2

Table 6 ANOVA output for log transformation of yield. Analysis Variable: Yield

Treatment	N	Mean	SD	Minimum	Maximum
Calcium Carbonate	7	58.1	36.4	8	98
Calcium Nitrate	7	262.9	390	22	1107
Control	7	51.9	36.2	1	118
Potassium Carbonate	7	55	36.4	6	100
Potassium Nitrate	7	97	38	48	153
Oyster Shells	7	79.1	68.8	11	218

R^2	CV	Mean (log of yield)
0.178	27.12	4.04

Figure 22 (left) Adult pepper weevil lays its eggs in the bud of the flower.

Figure 23 (right) Pepper weevil larva inside chili pepper. As the fruit grows the eggs hatch inside the pepper, cause damage and eventually fall off.

Figure 21 and Table 5 demonstrate raw data for pepper yield (fresh weight in grams). Fertilizer treatment effect was seen with $Ca(NO_3)_2$ with over two thousand grams of pepper. However the data for original yield was highly skewed towards the right; low yields were recorded due to pest damage caused by pepper weevil. Therefore the yield was transformed to normal by taking the logarithm of the yield value. The ANOVA determined a p-value > 0.05, indicating that the null hypothesis is not rejected and the variances for the treatments are equal. In other words there is a treatment effect, however it is not significant. Pepper yield was not affected by pH, but clearly fertilizer can increase yield.

According to Table 5, $Ca(NO_3)_2$ treatment resulted in the highest yield overall because of the fertilizer effect with excess nitrates readily available in the system (from nitrification and treatment application). Both potassium treatments had no significant difference in yield and are very similar to the control. The calcium treatments (both carbonate and nitrate) had higher yields than the control, most likely due to the excess calcium and nitrate in the system. From Figure 21 it can be concluded that pepper yield was not affected by pH. Thus, even without the pH factor, fertilizer input can help increase pepper yield.

Table 7 Plant tissue analysis from Agriculture Diagnostic Services Center (ADSC)

ITEM	Sample Lab No.	Description	Anal. Code	% N	% P	% K	% Ca	% Mg	% Na	% S	ug/g Fe	ug/g Mn	ug/g Zn	ug/g Cu	ug/g B	ug/g Mo	ug/g Al
1	151-0434		T1,2	5.87	0.26	1.82	1.31	1.22	0.13		43	161	64	6	62		

Table 8 Water sample analysis from ADSC

ITEM	Sample Lab No.	Description	Anal. Code	% N	% P	% K	% Ca	% Mg	% Na	% S	ug/g Fe	ug/g Mn	ug/g Zn	ug/g Cu	ug/g B	ug/g Mo	ug/g Al
1	151-0434		T1,2	5.87	0.26	1.82	1.31	1.22	0.13		43	161	64	6	62		

Statistical Analysis: Multivariable Analysis

Since there was no treatment effect from the one-way ANOVA analysis a multivariable analysis was conducted to see if there was any significant difference between the treatments and variables. The dependent variables included yield and the difference from final and initial weight (fish density). The independent variables consisted of treatment, pH and temperature. A multivariable analysis was conducted because two or more than two dependent variables exist. As seen in the table below, $p< 0.05$ means that the test results are significant and there is a difference amongst the treatments. An R^2 (coefficient of determination) value demonstrates how much the independent variable explains the dependent variable. Since R^2 value is less than 0.30 this shows that there is very low correlation between treatment and yield and for fish density.

Table 9 & 10 ANOVA output with dependent variable yield (left) and fish density (right)

Dependent variable:	**Yield**
R^2	0.2815
CV	24.05
Pr>F	<0.0001

Dependent variable: Fish density	
R^2	0.3259
CV	45.322
Pr>F	<0.0001

Linear Regression Analysis

The multivariate analysis demonstrated that there is a treatment effect for pepper yield and fish density. Therefore a linear regression analysis was conducted to see if there is any correlation between the pH and yield or fish grow. In both outputs the intercept are non-significant (the prediction is very low). From the analyses it can be concluded that there is a difference between treatments, however there is no correlation observed in the linear regression.

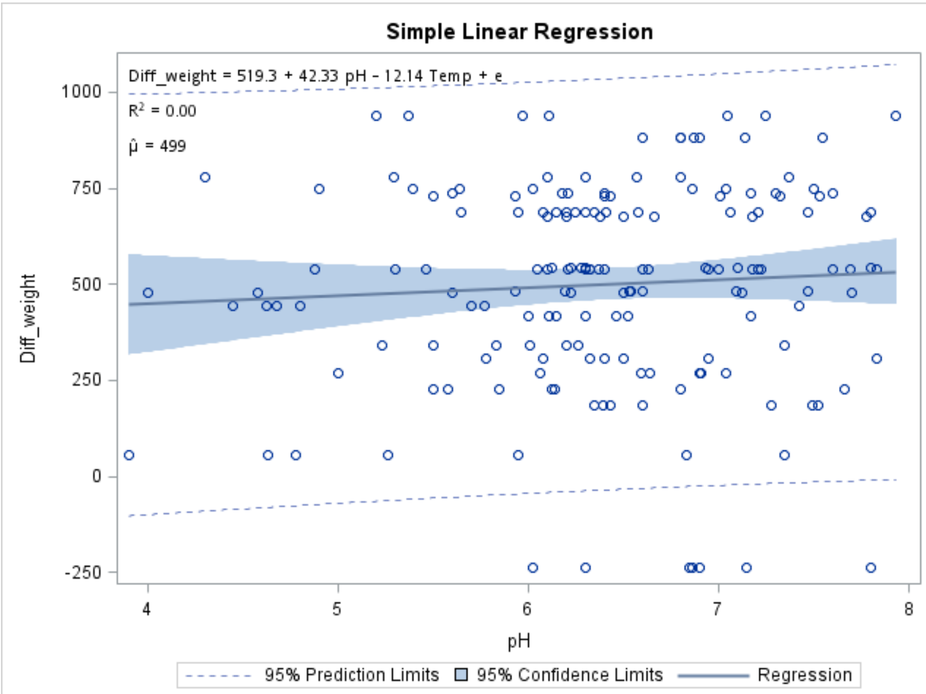


Figure 24 Linear regression analysis with yield as dependent variable and pH as the independent variable. R_2 value of 0.1 demonstrates a very low correlation between the variables.

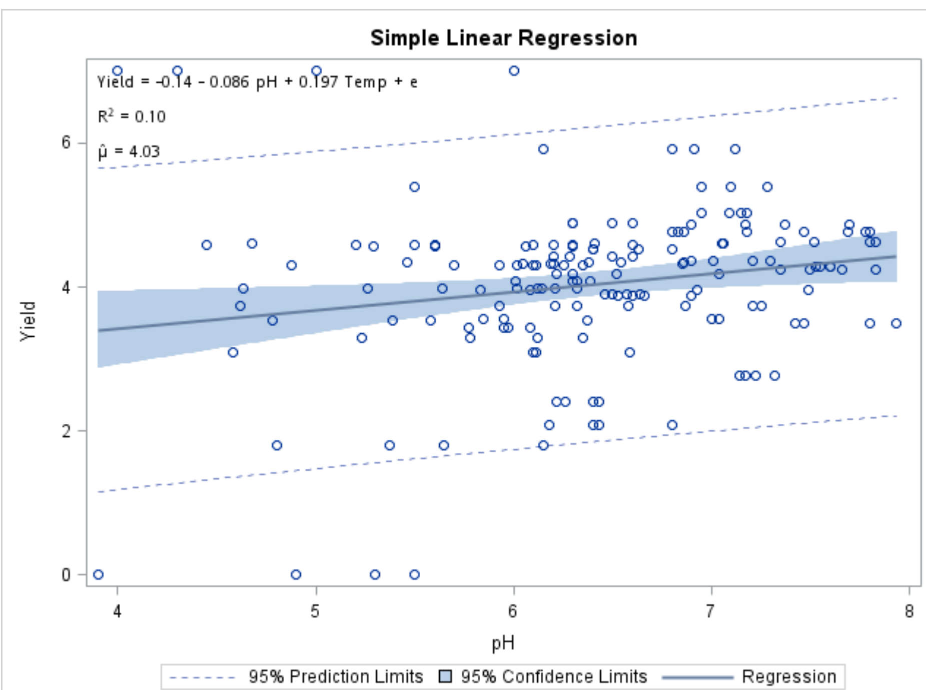


Figure 25 Linear regression analysis with fish weight (growth) as dependent variable and pH as independent variable. A R_2 value of 0.00 demonstrates no correlation between the pH and fish growth.

Capsaicinoid Quantification

Figure 28 reports the response of a red chili pepper sample treated with potassium nitrate. The following capsaicinoids were detected: noridihydrocapsaicin (NC) at 9 min, capsaicin (C) at 11 min, and dihydrocapsaicin (DC) at 11 min and 45 seconds. After the peaks were integrated and identified using the retention time of the standard, the capsaicinoids were quantified. Quantification uses the peak area to determine the concentration of a compound in the sample. The response of the unknown sample concentration was compared to the response of the known (standard) concentration to determine how much of the compound is present. To obtain a valid comparison for the unknown sample response to that of the known standard, the data was acquired and processed under identical conditions.

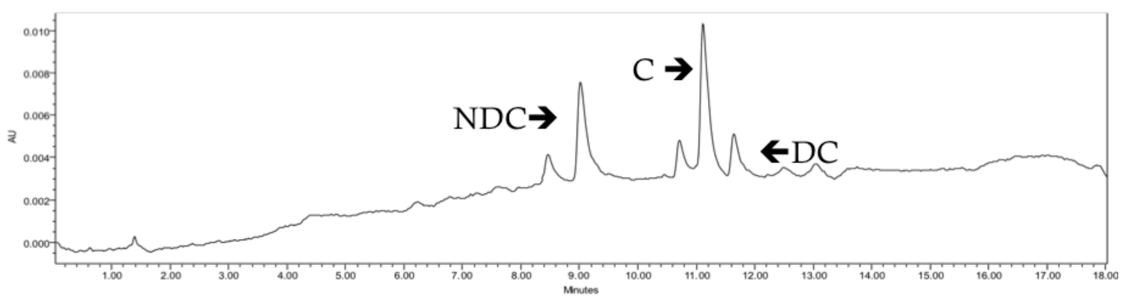


Figure 26 (above) HPLC Chromatogram of capsaicin (C) standard [0.05 mg/mL]. Detection set at 280 nm.

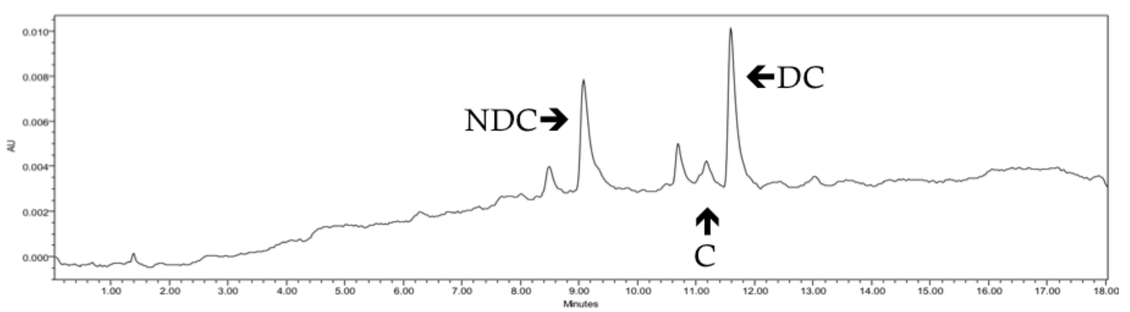


Figure 27 HPLC Chromatogram of dihydrocapsaicinoid (DC) standard [0.05 mg/mL]. Detection set at 280 nm.

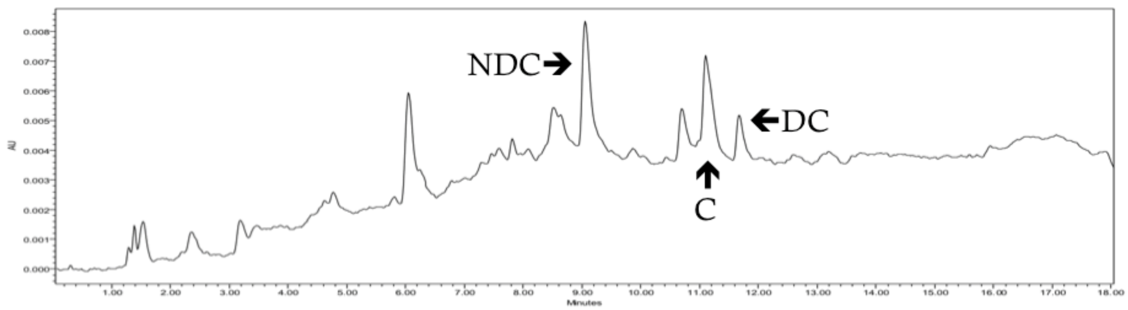


Figure 28 HPLC chromatogram of *Capsicum frutescens* 'Hawaiian Chili' shows baseline separation of capsaicin (major peak). A red chili pepper sample treated with KNO_3 with a concentration sample of [5.0 mg/mL]. Peak was identified with comparison to retention time of the standard compound (capsaicin). Peak area was used for quantitative calculations.

A calibration curve is a graphical representation of the amount and response data for a compound. The curve was constructed using the calibration (standard) solution of known concentration and measuring the peak area obtained. A line of best fit (regression line) was used to join the points of the curve obtained. A multi-level calibration using several calibration samples at different analyte concentrations was used. The peak area of each sample was used to calculate the concentration of capsaicin and dihydrocapsaicin using the equation from the line of best fit. Next the quantity of the concentration was calculated by multiplying the injection volume. Finally this quantity was multiplied by the dilution factor of 20. Dilution factor was calculated because only 50-uL of each sample was used for the analysis.

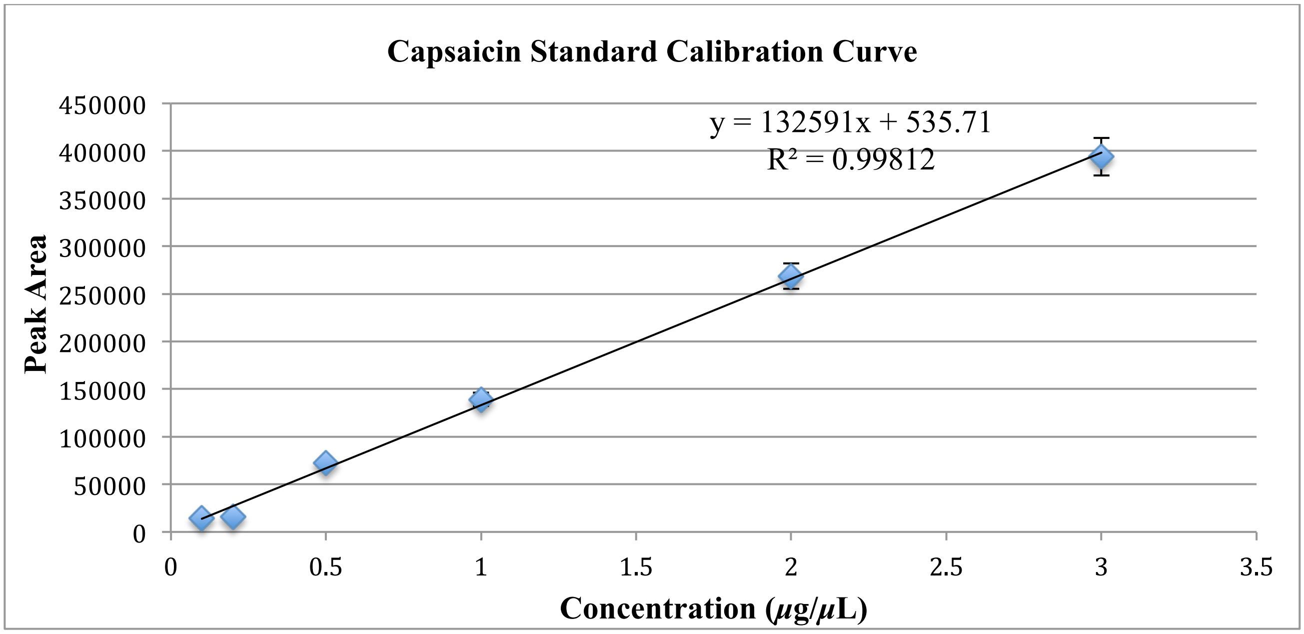


Figure 29 Calibration curve of capsaicin standard for quantitative analysis in HPLC. Triplicate injections of 10, 20, 40, and 60-μL were used at a concentration of 0.05 mg/mL.

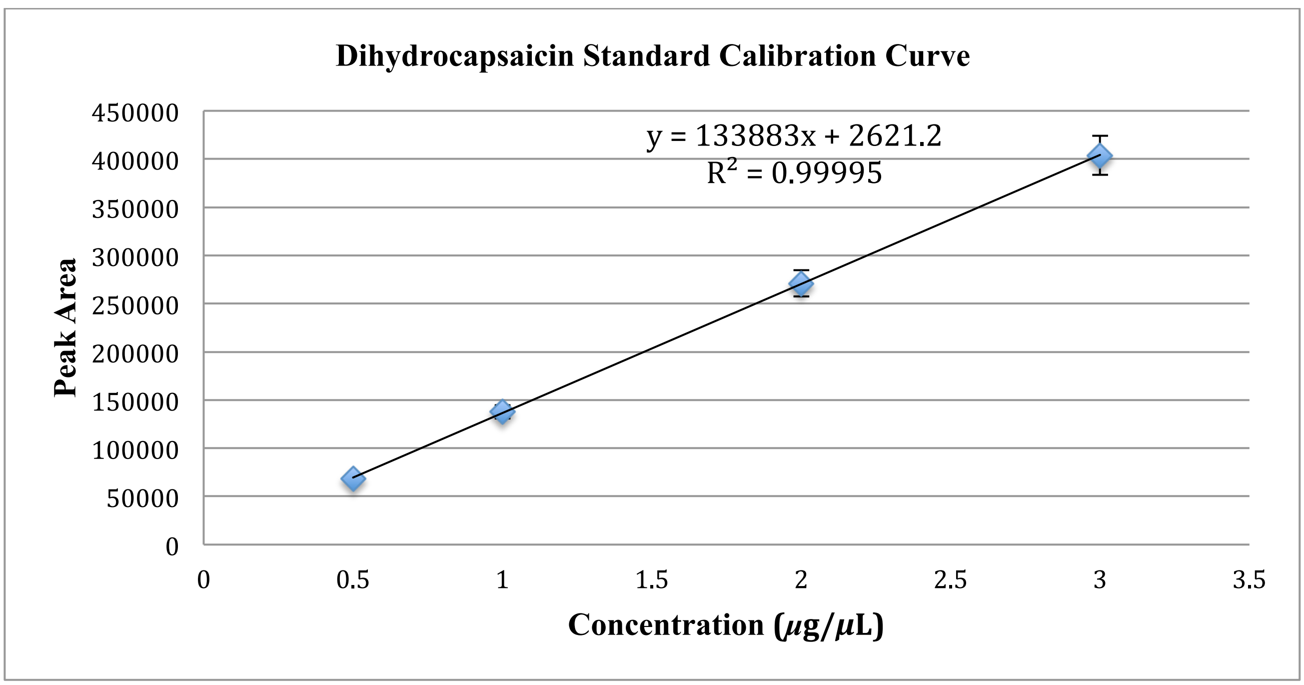


Figure 30 Calibration curve of dihydrocapsaicin standard for quantitative analysis in HPLC. Triplicate injections of 10, 20, 40, and 60-μL were used at a concentration of 0.05 mg/mL.

Table 11 Capsaicinoid content of red ripe chili peppers. Total capsaicinoids are made up of the sum of Capsaicin (C) and dihydrocapsaicin (DC) concentrations. Scoville heat units (SHU) were calculated by multiplying by a factor of 16.

Trt	Week	C (ppm)	DC (ppm)	Total	SHU	*	NDC
KNO_3	1	2678±160	758±135	3436	54976	-22%	↑
	2	459±34	127±15	586	9376	3.6%	↑
	3	394±20	72±26	466	7456	65%	↑
	4	794±29	239±42	1033	16528	95%	↑
K_2CO_3	1	274±105	54±13	328	5248	88%	↑
	2	275±80	87±33	362	5792	40%	↑
	3	----	-----	----	----	----	
	4	2122±119	760±78	2882	46112	87%	↓
$Ca(NO_3)_2$	1	642±103	110±67	752	12032	73%	↑
	2	347±29	62±17	409	6544	33%	↑
	3	453±35	138±9.3	591	9456	55%	↓
	4	849±13	312±12	849	13584	96%	↓
$CaCO_3$	1	3094±178	879±103	3973	63568	-41%	↑
	2	2086±366	540±170	2626	42016	-332%	↑
	3	3392±129	1218±51	4610	73760	-248%	↓
	4	1549±6.5	760±95	2309	36944	89%	↑
Oyster	1	529±151	132±59	661	10576	77%	↑
	2	393±65	83±40	476	7616	22%	↑
	3	537±29	206±5.7	742	11872	44%	↓
	4	368±58	123±9.3	491	7856	98%	↑
Control	1	2132±170	684±151	2816	45056		↑
	2	501±174	107±52	608	9728		↑
	3	1042±143	283±9.5	1325	21200		↑
	4	16206±984	5721±640	21927	350832		↑

---- Not determined (ND)
* Percentage of the control
NDC = Nordihydrocapsaicin

Trt	CV
KNO_3	**99.7**
K_2CO_3	**119.8**
$Ca(NO_3)_2$	**38.6**
$CaCO_3$	**31.0**
Oyster	**19.3**
Control	**151.3**

Table 12 (left) Coefficient of variation (CV) for capsaicin content of each treatment. CV demonstrates a dispersion of the

Table 13 Capsaicinoid content of mature green chili peppers. Total capsaicinoids are made up of the sum of Capsaicin (C) and dihydrocapsaicin (DC) concentrations. Scoville heat units (SHU) were calculated by multiplying by a factor of 16.

Trt	Week	C (ppm)	DC(ppm)	Total	SHU	*	NDC
KNO_3	1	1838±136	339±52	2177	34832	-261%	↓
	2	228±24	34±3.7	262	4192	54%	↑
	3	310±90	77±56	387	6192	----	↑
	4	----	----	----	----	----	
K_2CO_3	1	709±135	128±65	837	13392	-39%	↑
	2	1003±135	180±108	1183	18928	-106%	↑
	3	525±80	57±17.7	582	9328	---	↑
	4	764±45	248±37	1012	16192	76%	↑
$Ca(NO_3)_2$	1	318±34	60±13	378	6064	37%	↑
	2	485±75	148±40	633	10128	-11%	↑
	3	509±145	132±57	641	10256	----	↑
	4	----	----	----	----	----	
$CaCO_3$	1	281±30	85±16	366	5856	39%	↑
	2	1006±98	150±25	1156	18496	-102	↑
	3	512±115	358±56	870	13920	----	↑
	4	607±100	66±46	673	10768	84%	↑
Oyster	1	934±31	344±92	1278	20448	-112%	↑
	2	773±14	288±37	1061	16976	-85%	↑
	3	478±29	150±25	628	10048	----	↓
	4	----	----	----	----	----	
Control	1	472±55	131±52	603	9648		↑
	2	459±123	113±60	572	9168		↑
	3	----	----	----	----	----	
	4	3359±123	881±327	4240	67840		↑

---- Not determined (ND)
* Percentage of the control
NDC = Nordihydrocapsaicin

***Capsicum* species**	**Variety**	**ppm**	**SHU**
C. chinense	Habanero	20,000	320,000
C. frutescens	Hawaiian	14,000	224,000
C. frutescens	Thai	3,500	56,000
C. annuum	Jalapeno	2,000	32,000
C. annuum	Green bell	0	0

Table 14 (left) Capsaicinoid content in several chili peppers varieties [from Aza-Gonzalez et al. 2011 and Radovich et al. 2012]. A factor of 16 was used to convert μg/g to SHU.

The determination of the pungency is very important for consumers and for industrial purposes, since a defined value is required as an ingredient for processed food production. For this reason, pungency is considered subjective, and a universal scale from the American Spice Trade Association (ASTA) based on ppm of capsaicinoids is used. The ASTA units is a measure directly related to capsaicinoids amounts and can be easily converted approximately to the more common Scoville Heat Units by multiplying the amount of each capsaicinoid expressed in ppm by a calculated conversion factor (Ziino, 2009). The total pungency value of a given sample is obtained by adding the pungency values of the individual capsaicinoids. Capsaicinoid content can range from zero up to more than 300,000 Scoville Heat Units depending on genotype (DeWitt & Bosland, 1993).

The occurrence of capsaicinoids is highly variable as seen in Table 10. In this experiment there was no difference between the different maturity stages (red ripe and mature green) or treatments. In general capsaicin was more abundant than dihydrocapsaicin in red and green chilies. There is a difference between the treatments and the control however the buffer and fertilizer treatments had no effect on the capsaicin content. Instead the stresses observed actually lowered the capsaicinoid content. Unexpectedly, nordihydrocapsaicin content was higher than the other capsaicinoids. No standard was available for NDC therefore it could not be quantified.

Overall the treatment ranges are within the range of the control therefore treatment application had no effect on capsaicinoid content. The efficiency of treatments is ranked according to high to low total capsaicinoid content. For red chilies: control, $CaCO_3$, K_2NO_3, K_2CO_3, $Ca(NO_3)_2$, oyster; and for green chilies: control, K_2CO_3, $CaCO_3$, oyster, K_2NO_3, $Ca(NO_3)_2$. Overall best treatment for capsaicinoid content was the control, but $CaCO_3$ is more consistent in regard to week. Although oyster treatment is an efficient buffer for pH remediation, however in response to capsaicinoid content there is no effect. Compared to other studies, the following data shows that the Hawaiian chili peppers were as hot as a jalapeno due to environmental stress.

Discussion

Capsicum Yield

Vulnerability of capsicum occurred due to a multitude of abiotic and biotic stresses that restricted their potential yield. Abiotic factors that significantly diminish the yield and quality of peppers include extreme temperature, moisture, light, nutrients and pH among others (Ochoa-Alejo and Ramirez-Malagon, 2001). As well as biotic factors including susceptibility of peppers to various fungi, bacteria and viruses. Viruses are among the most important group of plant pathogens affecting the Capsicum production worldwide and cause catastrophic economic losses by reducing yield and compromising quality (Suzuki and Mori, 2003). Diseases caused by phytopathogenic fungi (*Phytophthora capsici, Fusarium and Rhizoctonia*) are also important in yield reduction of chili peppers (Egea et al., 2002). More importantly, flowering to fruit stage is critical for yield development (Jaimez et al., 2000). In this experiment pest damage targeted the peppers by laying their eggs within the flower bud, reducing the yield. Integrated pest management combined with improved agricultural practices can contribute to dramatic improvements in Capsicum crops upgrading both quality and yield. However in this experiment more action was required to prevent pest damage. An alternative would be to enclose the grow beds with a physical barrier. This minimizes pest exposure and additional maintenance tasks. Also when planting seedlings, must make sure extra seeds are planted to compensate for pest damage.

Variability of Capsaicinoids

Capsaicinoids are the alkaloids in hot peppers that are affected by a genetic and environment interaction. More importantly certain environmental factors or stressors, such as osmotic, nutrient, and pH stress, can be exploited to increase capsaicinoid content within the peppers. An investigation in Thailand identified the responses of capsaicinoid accumulation in hot pepper cultivars under drought stress conditions. Their findings were consistent with Estrada et al., 1999 in that this behavior of capsaicinoid production in pepper plants was previously observed when plants were subject to water deficiency. However the occurrence of capsaicinoids is highly variable, and strongly depends on cultivar, but also on other parameters such as ripening stage, season, and irrigation (Reyes-Escogido et al., 2011). Differences in pungency of fruits from the same plant have been reported depending on the moment of harvest (Kirschbaum-Titze et al, 2002), and also on the part of the plant from which the berry is picked. The distance from the base and the stem seem to influence the degree of pungency (Mueller-Seitz, 2008). Nonetheless, chili genotypes exhibit wide variation in capsaicinoid accumulation in response to genetic and more so with environmental factors.

Capsaicin Biosynthesis in Plants

Given the economic and agricultural importance of capsaicin, surprisingly little data exists on the genetics of this compound's biosynthesis. The capsaicinoid biosynthetic pathway has been established, but the enzymes and genes participating in this process have not been extensively studied or characterized. Capsaicinoids are synthesized through the convergence of two biosynthetic pathways: the phenylpropanoids and the branched-chain fatty acid pathways, which provide the precursors phenylalanine, and valine or leucine, respectively. Capsaicinoid biosynthesis and accumulation is a genetically determined trait in chili pepper fruits as different cultivars exhibit differences in pungency; furthermore, this characteristic is also developmentally and environmentally regulated (Aza-Gonzalex, 2000). Genetic and molecular approaches have also contributed to the knowledge of this biosynthetic pathway; however, more studies are necessary for a better understanding of the regulatory process that accounts for different accumulation levels of capsaicinoids in pepper fruits, especially in regards to environmental conditions.

Manipulation of Pungency

Capsaicinoids are important in the food and pharmaceutical industries. For this reason, a number of researchers are engaged in improving their production by manipulating chili plant cultivation conditions and alternative methods such as cell or tissue culture (Escogido-2011). Recently, it has been reported that an increase in antioxidant constituents of peppers occurs by applying nitrophenolate in the irrigation system (Serrano et al. 2010), in which the highest antioxidant activity was found in red maturity stage (Khan, 2014). Research aimed at increasing or improving pungent compound production has revealed that hydric stress increases capsaicinoid levels because water deficit affects the phenylpropanoid pathway. Hydric stress also increases capsaicin levels by raising activity of the enzymes phenylalanine ammonia-lyase (PAL), cinnamic acid-4-hydroxylase (C4H) and capsaicin synthase, all involved in capsaicin biosynthesis. These results suggest the possibility of controlling capsaicin synthesis in the plant by manipulating substrate concentrations and water availability, which would be a cost-effective, viable alternative for increasing capsaicin production. Capsaicinoids production in cells, tissue and organ cultures have been attempted in the recent years to increase capsaicinoid accumulation. Recent findings demonstrate that the manipulation of culture strategies such as osmotic stress, nutrient stress, or pH stress enhances the accumulation of capsaicinoids. Osmotic stress using NaCl resulted in the maximum capsaicinoid accumulation (Kehei et al, 2013). Studies using cultures derived from placenta of Capsicum annuum L. which were tried with 100% nitrogen stress increased capsaicin level by 9.8 fold of that in control after 15 days of sub-culturing (Varindra and Gosal, 2009). A study revealed that pH also enhanced capsaicin accumulation: in suspension cultures at pH 6 on day 15 enhanced accumulation of capsaicin. (Kehie et al. 2013). These in vitro studies on osmotic, nutrient and pH stress provide insight for strategies to increase capsaicinoids in the field.

Summary

Secondary metabolites such as capsaicinoids are involved in protective function in response to both biotic and abiotic stress conditions. The concentrations of various secondary plant products are strongly dependent on the growing conditions that impact their metabolic pathways. This experiment demonstrated that the environment cammn have a greater effect on capsaicinoid levels than genotype. Exposure to salt stress in soil or water is one of the major stresses and can severely limit plant growth and productivity (Ramakrishna, 2011), resulting in decrease of specific secondary metabolites in plants. Although in vitro studies demonstrate that osmotic stress increases capsaicinoids, in the field nitrogen stress works best especially in aquaponics. Drought stress was also one of the most significant abiotic stress that affected plant growth and development. Therefore, the response, in terms of capsaicinoid content, of peppers (*Capsicum* frutescens) cultivated in stressful aquaponic conditions was highly variable primarily due to environmental conditions. Information pertaining to peppers grown in stressful aquaponic conditions for capsaicinoid content is limited. Thus the null hypothesis was accepted: once treatment application is applied the capsaicinoid content decreases. The objective of this analysis was to measure the response of capsaicin and dihydrocapsaicin for total capsaicinoid content. It was observed that nordihydrocapsaicin had elevated concentrations within the samples however there was no standard available at the time therefore it could not be quantified. Overall the higher concentrations of nordihydrocapsaicin indicated that capsaicin congeners are inhibited due to excessive nitrates within the system water.

Chapter 5

Conclusion

Trends of the American Diet

The Food of Paradise

Local food in Hawaii is Hawaii's food, a society with one of the richest culinary heritages in the US. Today Hawaii's foods are imperfectly adjusted to the soil and climate of the Islands. Nowhere has this been more pronounced than in Hawaii, the most isolated of all the islands and the most ethnically mixed. A land originally largely barren of edible plants and animals; peopled by three successive waves of migrants struggling to feed themselves and their families and eventually creating a creole food based on imported foodstuffs.

Since the Pacific Islands are so isolated, anthropologists see them as natural laboratories for studying how societies evolve. Hawaii has a range of ethnic groups, no majority, and a 50% intermarriage rate; sociologists turn to it to see how multicultural societies develop. Since Hawaii's people, with their diverse tongues, have created "pidgin" (Hawaii English dialect) to make communication possible, linguists use the Islands to study how new languages are created. Nowhere else in the world are Pacific, Asian, and Caucasian food traditions in such close contact; in which a new fusion is being created year by year. Also the University plays an essential role for research and development from distant galaxies to the ocean depths and everywhere else in between. Hawaii is just as fascinating to anyone interested in foods, science and its history.

In a society that has little in common except the language 'pidgin', where neither religion, literature, art, music, social customs, nor a long shared history provide a common ground, Local Food serves as an essential, basis that glues the diverse peoples of Hawaii together. Sharing food is so important because this is one way individuals can make contact with their neighbors. Recognizing this use of food as a common language drives home the point that food sustains more than the body that it also sustains cultures. What makes people in Hawaii feel they belong is that they share Local Food.

A cuisine in Hawaii that attracts attention is Hawaii Regional Cuisine (HRC). Named in 1992, when a group of chefs – Sam Choy, Roger Dikon, Amy Ferguson Ota, Mark Ellman, Beverly Gannon, Jean-Marie Josselin, George Mavrothalassitis, Peter Merriman, Philippe Padovani, Gary Strehl, Alan Wong, and Roy Yamaguchi – incorporated to sponsor a cookbook to be sold for charity. An integral part of this movement was the local farmer: Dean Okimoto (Nalo Farms, Inc.). The group has taken a tired international upmarket restaurant cuisine, based on imported products, and replaced it with a cuisine based on foods grown in the Islands. "Boutique farmers" provide fresh radicchio, sweet onions, red ripe strawberries, a range of European and Southeast Asian herbs, and the superb Hawaii Vintage Chocolate; fishermen bring in the best of their catch; and the hunters supply wild boar and venison from Hawaii's mountains.

Food through the ages has been a counterpoint between the food of the chefs, with its access to whatever ingredients money could command and time for complicated preparations, and the food of the people, put together with whatever the budget could rise to and with whatever time was available. HRC was created by a force quite different from those that drive Local Food. The chefs were catering to well-heeled customers from around the world prepared to spend money on eating out; the Locals were catering to a specific Local taste. The chefs were influenced by international nouvelle cuisine while trying to create their own identity by incorporating ingredients and traditions from Hawaii; Locals were influenced by what could be put together with materials available in the supermarket at reasonable cost. The chefs developed recipes that assumed kitchen help and efficient grills; Locals wanted recipes that could be whipped in 20 minutes in a tiny high-rise kitchen surrounded by three hungry children. And the chefs had access to locally grown strawberries and chocolate and venison, while locals had woolly strawberries from California, Hershey Bars, and SPAM. But although the forces creating cross-fertilization can be nothing but mutually beneficial, creating a firm regional base for the cuisine of the restaurants and increasing sophistication for the cuisine of the home and the street.

America's Healthcare System

Animal, meat, dairy products are undermining the health of the American people. With Obamacare, costs are out of control and there are issues about who should pay. And yet there is very little conversation about governmental policies that encourage healthier food choices. Right now USDA and health authorities are telling us to eat more fruits and vegetables. But USDA is spending billions every year subsidizing GMO corn and soy that are then fed to animals, which lowers the price of meats and dairy products. USDA is spending nothing to subsidize production of fruits and vegetables, the foods they are telling us we should be eating more of. So what sense does it make for us as taxpayers to be subsiding the foods that are driving up healthcare costs and that are making us sick and fat. Shouldn’t we as taxpayers subsidize the foods that are consistent with our health and well-being? There are people that don’t want it to change because they profit from the sales of meats and processed foods. But that’s okay. The tobacco industry didn’t want us to lower smoking rates. There are other things these people can do to make a good living by producing products that are good for people.

Environmental Impact of Animal Agriculture

Industrial meat production and factory farming is a violation of the bond between humans and animals. The harsh truth is if you eat meat, eggs, and dairy you're essentially eating your way into extinction. It wasn’t always this way; to understand how we got here we need to look back. Before the industrial revolution the way we farm changed drastically. Production changed from small local farms to large factory farms. Often indoor in high densities, with one single goal: to produce the most amount of meat with as little cost as possible. This system of factory farming emerged to feed the population of 1 billion in early 1800s and 6 million by early 2000. And thanks to government subsidies that encourage over-production especially in America and Western Europe, this ended up reducing the price of meat. All this seems like progress on the surface but it all came at significant cost to the planet as well as our health.

Animal agriculture is the single most destructive industry responsible for the current ecological crisis. It occupies almost half the land resources, uses majority of fresh water and drives more greenhouse gas emissions than the entire transportation sector combined. It gets worse: air and water pollution, land degradation, deforestation the list goes on.

This massive industrial system of food is devastating our ecosystem. If we continue on this path in order to feed the 9-billion population by 2050 we will deplete our planet's resources. Thus plant-based eating is an important topic to discuss especially for the environment.

The world produces enough calories to feed 10-billion but still people are going hungry. Majority of the calories of our staple crops are going to animals. 70% of grains in the US go to cattle for feed. This is not only an environmental crisis it's a humanitarian crisis as well. The grains fed to cattle are not even their natural food source. This is done because the system is designed to produce the maximum amount of product at the fraction of the cost. Basically to fatten up animals to grow quickly as possible for the economic value of the farm. This is a prime example of soy plantation production for cattle feed. All this is happening to feed this massive appetite we have in the western world.

Oxford Study on Global Sustainability

A scientific study published in the proceedings of the National Academy of Sciences was conducted on the effectiveness of certain strategies regarding sustainability. One factor had the most impact, spanning not only water conservation, and resulting in the most cost savings across economies, and helping the planet, but also ameliorating human health, saving human lives. The scientific results concluded that the number one factor with significant impact was altering one's diet to a vegan diet. When a vegan diet was tested, it resulted in saving the most lives, making the greatest economic impact, and it resulted in the most profound benefits to environmental factors.

The researchers checked the impact if people chose to follow various diets: meat diet, vegetarian diet, vegan diet, etc. The diet containing any meat was scientifically found to have the worst results for the environment, against human health, and regarding costs to people eating meat diets; meat resulted in the unfortunate findings that those that continue to follow a diet with meat would result in 5.1 up to 8.1 million human deaths (Springmann et al., 2016). Moving away from a standard meat-centric diet and adding more fruits and vegetables, though showing improvement, still was not optimal. The addition of more fruits & vegetables and less meat resulted in the savings of 5.1 million lives. Adopting a vegetarian diet, one that still includes animal products such as milk and eggs was 'better', but still not good enough and not optimal. For example, compared to a diet with meat, a more plant-based vegetarian diet resulted in saving 7.1 million lives (Springmann et al., 2016). The highest most profound and effective findings came when researchers tested a 100% vegan diet. This resulted in saving 8.1 million lives, the most of any of the factors tested in the study. A vegan diet was found to have the most environmental impact, generated the least greenhouse gasses, saved the most water, and saved the most human lives. Affirming once again that a vegan diet was the only optimal result with regard to cost, health, and environmental sustainability.

The Growth of the Plant-Based Movement

As mentioned above the diet that causes the least amount of harm, ecologically earth friendly, and has the highest quality of life and health is the vegan diet. This diet allows for a socially responsible society. It takes moral courage to break away from the traditional American diet because we are conditioned culturally to eat meat. Nonetheless the standard American diet is set. However, when we change our diet the results are dramatic and life affirming. So many things are fed when we are fed well and when the foods that we choose are in alignment with ourselves are also the kindest to the animals. This is a powerful combination of realities.

The most empowering impact one can have for the planet and for one's own health is to change to adopt a plant-based diet. The strategic triad of plant-based eating benefits includes: good health, good for the environment, and essentially a more humanitarian way to live. Just by changing your diet you address all three of these factors. But some individuals like meat and do not want to give it up. So in reality the general population is struggling and there is tension. The fact that those things are true is a source of guilt. So there is an interesting situation. For the movement to continue in the right direction vegan activists want the number of vegans to increase. That's how you measure success of the movement; however, this is not likely. In 10-15 years how many individuals will be vegan? Most likely zero or close to zero. But what are the odds that half the meals eaten in the country will be vegan? That's more of a realistic goal, which is doable, achievable and possibly can surpass that. Becoming more plant-based or plant-strong, instead of imposing the vegan diet onto others. There is no need to make people feel excluded if they eat animal products; just need people to eat less of it. As we all eat less, more of us will eat none. What will happen is we will be a healthier people, with a more positive impact on the environment in which our relationship with animals will start to improve and remember the connection with the web of life. In another sense, be protectors not predators of the planet.

Organic food and plant-based lifestyle is now mainstream. Making healthy food choices that are socially and ecologically responsible are becoming increasingly trendy. Al Gore, Bill and Chelsea Clinton are vegans. Prominent people, celebrities and athletes are vegan. It’s becoming trendy because of the reality of how important this issue is. As mentioned above in the Oxford study, the food movement has been scientifically validated. A new healthcare model involves integrating food, fitness, and lifestyle. For example, the Ornish program is now covered by insurance. When you eat a healthier diet not only do you lower your disease risk but also you feel better. All of your senses become more acute, your taste buds work better. To put it in a stark and eloquent way: choose a healthy diet because health is pleasure and sickness is not!

Change through social media.

A collective solution is eminent for our current food system and for the sake of our environment. Not only is this an environmental issue it's about the future of humanity. In order to feed the estimated 9 billion in 2050 we need to produce more food sustainably. For one of the most important issues of our time, the power of social media is one way to resolve this. Deliver the message using the Internet. The idea or message is simple: changing the way you live and eat can save the world; the fact that one’s everyday choices are in someway contributing to the ecological crisis. This plant-based movement allows individuals to make healthier food choices that have a positive impact on the planet. This is an exclusive movement that lays down the path and you do the rest at your own pace. Empowering individuals to make planet friendly food decisions and support innovation in the food industry are the most tangible solutions we have in our current environmental crisis.

Currently most of the focus is on problems such as climate change and issues with the food industry. There is very little focus on solutions; no information out there presented in a way that's engaging and entertaining; not always talking about the problems but offering positive solutions as well. An online community along with Internet education such as foodrevolution.org and onegreenplanet.org are online tools that help individuals learn and take action in their lives. These digital media platforms help proponents learn and take action in their lives on behalf of what they love, including their health. It’s a new world to do this online, in the past newspapers and colleges are essential institutions for new knowledge, but in order to reach the masses and have a significant impact the internet is key. The purpose of these digital media platforms is to help the people make informed decisions about what they eat. Essentially building an active plant based nutrition support group. They show the path we are on with our current food system (impact of animal agriculture on environment) and provide simple solutions (such as plant based recipes and cooking tips) one can do daily and/or multiple times a day.

This movement is spreading through media and using young people to build a global online community. The demographic of the readers are ages 18-35 but also skewed toward women 30-70 years of age. Mostly in the U.S. but it’s a global movement in which folks are interested in changing the way they live. They are inspiring people to change their lifestyles in regards to the way they eat to make a positive impact on the environment. Also using the power of the Internet, nutrition, and individual lifestyle choices to contribute to the movement and inspire more people to adopt change. The majority of them are meat eaters or vegetarians (not vegan) that can help one meal at a time. This work truly contributes to the well being of other human beings and the whole earth community.

Summary and Future Work

The goal of the proposed study is to understand the effects of buffers and fertilizer application in aquaponics and its influence on crop yield and quality. Therefore experiments were conducted to remediate pH, determine the treatment effect, and compare capsaicinoid content between the treatments. The hypothesis tested is that treatment application remediates pH while increasing capsaicinoid content was rejected.

There was no link determined between treatment and capsaicin content because the control contained higher levels of capsaicinoids compared to the rest of the treatments. Oyster shell is an efficient buffer for pH remediation, however it does not have an effect on capsaicin content. Although in vitro studies have proven that certain stressors can be applied to increase capsaicinoids, the current experiment contained numerous stressful conditions that contributed to reduced yield and quality. Environmental stressors included: pest damage, water stress, pH stress, nutrient deficiencies, and lack of physical space for roots within the system. Results from capsaicinoid content analysis demonstrate that environmental stressors reduced not only the yield but the quality of capsaicinoid content as well. The stressful conditions prevented the metabolism of the secondary metabolite, capsaicin but favored the metabolism of noridyhydricapsaicin (tertiary metabolite). From the analysis it can be concluded that the environment has more of an impact on capsaicinoid content than chili pepper cultivar.

To improve future studies there should be a continuation to find a relationship between harvest stage and capsaicinoid content with various fertilizer applications. To do this environmental components should be controlled with various inputs: 70% black shade cloth that allows wind to flow through (instead of clear white tent that accumulates heat); improve experimental design to incorporate efficient water flow throughout the system to prevent nutrient and osmotic stress (osmotic and nutrient stress in the field lead to reduced yield and productivity). Given the economic and agricultural importance of capsaicin, low production yield has led to development of new synthesis strategies (Escogido, 2011) including manipulation of growth conditions or addition of supplements to improve capsaicin biosynthesis in the plant. To manipulate capsaicinoid content in aquaponics other beneficial stressful conditions can be applied to the aquaponic system such as controlled nitrate stress. Simply adding more fish to the system or increasing feed input can do this, as well as changing the feed composition (protein, fat, etc.). Nonetheless, oyster shells are an efficient buffering agent however other natural sources of nutrient supplementation (potassium and iron) for plants should also be incorporated.

Reduce Production Cost with Aquaponics

Hot peppers are almost ubiquitous in the ethnic foods of Hawai'i; even Japanese and many Pacific Islanders have adopted them. Serving a bottle of chili pepper water is a common practice on Thai and Filipino dining tables. In Hawai'i, hot peppers are especially important in hot sauce and other value added products. However**,** local sauces are produced almost exclusively from imported peppers. Replacing imports of these high-value specialty crops from local growers allows better marketability for products while reducing production costs. Also small fruit peppers are preferred by the Asian and Pacific markets but labor cost is a major component of production for small peppers. In spite of this, genetic variability in fruit size within "types" can be exploited to reduce labor costs. In other words larger peppers such as Jalapeno and Serrano peppers require less labor compared to smaller varieties such as Hawaiian and Thai chilies types. Therefore, replacing imports and exploiting fruit size can help reduce production cost of pepper grown in aquaponics.

It is difficult for growers to rely primarily on pepper production for their business model, but they can help a small farmer diversify and help reduce reliance on imports. Researchers at University of Hawaii hope that several local farmers will pick up their efforts and try to grow the most prolific peppers commercially, widening the pepper market in Hawaii's local market. Chili peppers are easier to grow in Hawaii than mild green bell peppers. Among the chili peppers that grow well are small fruit varieties including Super chili, Hawaiian chili, and University of Hawaii Waialua. Production of certain peppers in aquaponics is still elementary due to size requirements such as space for roots and height but certain varieties are suitable (Super chili). Nonetheless, opportunity exists to create and expand niche markets for small growers in Hawai'i and elsewhere.

Global Change with Aquaponics

The U.S. is blessed with an abundance of fertile soil in most states. However, countries like Australia, New Zealand, Israel and Holland rely on their not-so-fertile soil to act like a foundation base for hydroponic greenhouses and aquaponic systems to produce enough vegetables and fish to feed their people (Bernstein, 2007). The two main drivers of the projected increase in global demand for food in the next forty years: global population growth and increasing standards of living for developing nations. Climate change is another major threat to biodiversity since plants are extremely sensitive to such changes, and do not generally adapt quickly (Ramakrishna, 2011). Now, with the pressure to produce more food, even countries with abundant areas of fertile soil are looking at both hydroponics and aquaponics to produce fish or food crops both in a faster growth cycle and in more volume in a given space. Given the ecological and economic viability of aquaponics, it can be part of the solution for our future food supply demands.

Along with this demand, the local movement demands locally grown, fresh produce in meals they eat, both at home and in restaurants. Health-conscious consumers also want an increasing quality of food that is local and sustainably grown not just for a healthy lifestyle but also for a healthier environment. Aquaponics fills the demand for these consumers. This technology can be used to raise fish and fresh produce at any scale, from very large commercial systems to very small personal setups and everything in between. Whatever the size, all aquaponics systems use the same concepts and technology.

The integration of agriculture and aquaculture has been practiced globally in one form or another by many indigenous cultures throughout history. Modern aquaponics is an agricultural technology that continues to gain popularity as a method for food production, both commercially and small-scale, backyards systems. Compared to soil-based production systems, the many benefits that aquaponics techniques offer include: minimal environmental impact, low resource requirements, and efficient and high quality production on marginal agricultural lands. Also reducing electricity cost through renewable energy can be achieved in equatorial areas by harnessing hydro or solar energy. In addition to this there is an increasing attention being given towards reducing the production cost of agricultural crops. In aquaponics, fish waste is the alternative fertilizer source that reduces fertilizer inputs, making agricultural practices more sustainable.

Thesis in a nutshell

The purpose of the experiment was to determine treatment effect in aquaponics in relation to water quality, fish growth, plant yield, and capsaicinoid content.

It was proven that when nitrification continues in aquaponics, then pH becomes acidic. Once pH is below 5, then various treatment applications remediates pH. Unexpectedly, once treatment application is applied, the capsaicinoid content decreased. In all, highest pepper yield was observed in calcium nitrate and calcium carbonate treatments. The capsaicinoid analysis demonstrated that there was no treatment effect, peppers had low capsaicinoid content, and there was high variation overall. From the aquaponic analysis, ammonia and nitrite toxicity lowered fish growth in the oyster and calcium carbonate treatments. Surprisingly high nitrates (in calcium nitrate treatment) had no affect on fish growth. And potassium carbonate is not an efficient buffer, however calcium carbonate and oyster shells are adequate.

References

1. Ako, Harry. 2014. How to build and operate a simple small-to-large scale aquaponics system. College of Tropical Agriculture and Human Resources. University of Hawaii at Manoa, Honolulu, Hawaii. Center for Tropical and Subtropical Agriculture Publication #161
2. Alexandratos, N.; Bruinsma, J. *WORLD AGRICULTURE TOWARDS 2030/2050: The 2012 Revision*; FAO, Agricultural Development Economics Division: Rome, Italy, 2012.
3. Al-Hafedh, Y.S.; Alam, A.; Alam, M.A. Performance of plastic biofilter media with different configuration in a water recirculation system for the culture of Nile tilapia (*Oreochromis niloticus*). *Aquac. Eng.* 2003, *29*, 139–154.
4. Antoniou, P., J. Hamilton, B. Koopman, R. Jain, B. Holloway, G. Lyberatos, and S. A. Svoronos. 1990. Effect of temperature and pH on the effective maxi- mum specific growth rate of nitrifying bacteria. Water Res. 24:97-101.
5. Appendino G: Capsaicin and capsaicinoids. In: *Modern Alkaloids* (Fattorusso E, Taglialatela-Scafati O, eds.). Wiley-VCH, Weinheim, 2008, pp. 73-109.
6. *Aquaculture to remain fastest growing food sector.* The Fish Site. July 2014. Web: www.thefishsite.com/fishnews/23620/aquaculture-to-remain-fastest-growing-food-sector/
7. Aza-Gonzalez, C., H. G. Nunez-Palenius, N. Ochoa-Alejo. Molecular biology of capsaicinoid biosynthesis in chili pepper (*Capsicum* spp.). *Plant Cell Rep*. 2011, 30:695-706.

8. Barbero GF, Palma M, Barroso CG: Pressurized liquid extraction of capsaicinoids from peppers. J Agric Food Chem 2006;54: 3231–3236.
9. Bernstein S. 2011. Aquaponic Gardening: A step-by-step guide to raising vegetables and fish together; New Society Publishers: Gabriola Island, BC, Canada; p. 256.
10. Beyers T. 2004. Cost-benefit analysis of the integration of a RAS in a heated tomato greenhouse. Paper Presented at Aquaculture Europe 2014, October 14-17, Donostia-San Sebastian, Spain.
11. Brunty, J. L. 1995. Biological filtration for ornamental fish production and factors affecting total ammonia nitrogen and nitrite removal rates. M.S. Thesis, Univ. of Fla., Gainesville.
12. Conservation [Internet]. 2015. United States Department of Agriculture; [updated 2015 Jan 9; cited 2015 Oct 8]. Available from: www.usda.gov/wps/portal/usda/usdahome?navid=conservation
13. Collins, M.D., L.M. Wasmund, P.W. Bosland. Improved method for quantifying capsaicinoids in *Capsicum* using high-performance liquid chromatography. *HortScience.* 1995, 30(1): 137-139.

14. Colt, J. E. and G. Tchobanoglous. 1976. Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. Aquaculture 8:209-224.

15. DeWitt, D. & P.W. Bosland, 1993. The Pepper Garden. Ten Speed Press, Berkeley,

California.

16. Dybas CL. 2005. Dead zones spreading in world oceans. Bioscience 55, 552-557
17. Dimitri C, Oberholtzer L. 2009. USDA-Marketing U.S. Organic Foods, Recent Trends from farms to consumers. Economic Research Service; Economic Bulletin #58 Sept. 2009. http://www.ers.usda.gov/media/185272/eib58_1_.pdf
18. Howitt R, MacEwan D, Medellin-Azuara J, Lund J, Sumner D. 2015. Economic Analysis of the 2015 Drought for California Agriculture [Internet]. [cited 2015 Nov 3]; UC Davis Center for Watershed Sciences. Available from: http://watershed.ucdavis.edu/files/biblio/Final_Drought%20Report_08182015_FullReport_withAppendices.pdf
19. Radovich TJ, Cox LJ, Hollyer JR. 2009. Overview of organic food crop systems in Hawaii. CTAHR, UHM. Sustainable Agriculture Nov 2009 (SA-3)
20. Food and Agriculture Organization. 2015. FAO Global Aquaculture Production Database updated to 2013-Summary information [Internet]. 2013. [cited 2015 July 27]; Food and Agriculture Organization of the United Nations – Fisheries and Aquaculture Department. Available from: http://www.fao.org/fishery/statistics/en
21. Godfray HC, Beddington JR, Crute IR, Haddad L, Lawrence D, Munir JF, Pretty J, Robinson S, Thomas SM, Toulmin C. 2010. Food Security: the challenge of feeding 9 billion people. Science 327, 812-818. DOI: 10.1126/science.1185383
22. National Oceanic and Atmospheric Administration. 2007. NOA 10-year plan for Marine Aquaculture. Available from: www.nmfs.noaa.gov/aquaculture/docs/policy/final_noaa_10_yr_plan.pdf
23. Rakocy, JE. 1989. Island Perspectives; Virgin Islands agricultural experiment station: Saint Croix, VI, USA. P. 5-10.
24. Turcios AE, Papenbrock J. 2014. Sustainable Treatment of Aquaculture Effluent – What can we learn from the past for the future? Sustainability, 6, 836-856.
25. Goddek S, Delaide B, Mankasingh U, Ragnarsdottir KV, Jijakli H, Thorarinsdottir R. 2015. Review: Challenges of sustainable and commercial aquaponics. Sustainability 7, 4199 – 4224. DOI: 10.3390/su7044199.
26. Tyson RV, Simonne EH, Davis M, Lamb EM, White JM, Treadwell DD. 2007. Effect of nutrient solution, nitrate-nitrogen concentration and pH on nitrification rate in perlite medium. Journal of Plant Nutrition, 30, no. 4-6 (2007): 901-13.
27. Tokunaga K, Tamaru C, Ako H, Leung P. 2015. Economics of small-scale commercial aquaponics in Hawaii. Journal of World Aquaculture Society. Vol 46, No. 1 DOI: 10.1111/jwas.12173
28. Resh HM, 2004. Hydroponic Food Production. 6th Ed. New Concept Press Publising Co., Mahwah, NJ.
29. Tyson R.V., Simmone E.H., White J.M., Lamb E.M. 2004. Reconciling water quality parameters impacting nitrification in aquaponics: The pH levels. Proc. Fla. State Hort. Soc. 117:79-83. 2004.
30. Edwards p. 2015. Review: Aquaculture environment interactions: Past, present and likely future trends. Elsevier Aquaculture 2015 Feb; 447 (2015) 2-14.
31. Rakocy, J. E. 1999. Aquaculture engineering—the status of aquaponics, part1. Aquaculture Magazine 25(4):83-88.

32. Timmons, M. B., J. M. Ebeling, F. W. Wheaton, S. T. Summerfelt, and B. J. Vinci. 2002. Recirculating aquaculture systems, 2nd Edition. Northeast Reg. Aquaculture Center Publ. No. 01-002.
33. EPA. 2015. Overview of Greenhouse Gases. United States Environmental Protection Agency, Washington, DC, USA. Available from: http://www3.epa.gov/climatechange/ghgemissions/gases/n2o.html → EPA
34. Hu, Z., Lee, J.W., Chandran, K., Kim, S., Brotto, A.C., Khanal, S.K., Effect of plant species on nitrogen recovery in aquaponics, Bioresource Technology (2015), doi: http://dx.doi.org/10.1016/ j.biortech.2015.01.013
35. Hargreaves, J.A., 1998. Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166 (3-4), 181-212.
36. Molden, D. (Ed.), 2007. Water for Food, Water for Life, a Comprehensive Assessment of Water Management in Agriculture. International Water Management Institute, Colombo, p. 645 (Earthscan, London).
37. Martins, C.I.M.; Eding, E.H.; Verdegem, M.C.J.; Heinsbroek, L.T.N.; Schneider, O.; Blancheton, J.P.; D'Orbcastel, E.R.; Verreth, J.A.J. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquac. Eng.* 2010, *43*, 83–93.
38. Food and Agriculture Organization. 2016. The state of food insecurity in the world 2015. http://www.fao.org/hunger/key-messages/en/
39. Food and Agriculture Organization. 2012. Statistical Yearbook; FAO: Rome, Italy; p. 366
40. MEA. 2005. Ecosystems and human well-being: Synthesi; Millenium Ecosystems Assessment; Island Press: Washington DC, USA, p. 137
41. EPI. 2008. Global Assessment of closed system aquaculture. EcoPlan International: Vancouver, BC, Canada; p.79
42. VanRijin J. 2013. Waste treatment in recirculating aquaculture systems. Aquac. Eng. 53, 49-56
43. Schneider O, Sereti V, Eding EH, Verreth JAJ. 2005. Analysis of nutrient flows in integrated intensive aquaculture systems. Aquac. Eng. 32, 379-401
44. FAO. 2005. AQUASTAT Survey, Irrigation in Africa in Figures; FAO: Rome, Italy.
45. WWAP. 2012. The United Nations World Water Development Report 4: Managing water under uncertainty and risk; World Water Assessment Programme; United Nations Educational: Paris, France; p. 380
46. Talbot D. 2014. Desalination out of Desperation. Technology. www.technologyreview.com/featurestory/533446/desalination-out-of-desperation/
47. Loke M, Geslani C, Takenaka B, Leung PS. 2012. Seafood Consumption and supply sources in Hawaii, 2000-2009. Marine Fisheries Review 74: 44-51
48. *Irrigation & water use.* United States Department of Agriculture Economic Research Service. October 2015. Web: http://www.ers.usda.gov/topics/farm-practices-management/irrigation-water-use.aspx
49. Subasinghe, Rohana. Aquaculture topics and activities. State of world aquaculture. *FAO Fisheries and Aquaculture Department*. Rome. Updated 27 May 2005 (Cited 15 December 2015). Web. http://www.fao.org/fishery/topic/13540/en, http://www.fao.org/sof/sofia/index_en.htm.

50. UNFAO. Human development report. 2013. Web: http://hdr.undp.org/en/media/HD_2013_EN_complete.pdf
51. Hochmuth, G. 1991. Florida greenhouse vegetable production handbook. Univ. Fla. Extension Circ. SP 48, vol. 3.
52. Resh, H. M. 1998. Hydroponic food production, 5th ed. Woodbridge Press Publishing Co., Santa Barbara, Calif.
53. Harmon, T. 2001. A look at filtration in aquaponic systems: bead filters. Aquaponics J. 5(3):16-19.
54. McGee, M. and C. Cichra. 2000. Principles of water recirculation and filtration in aquaculture. Univ. Fla., Dept. Fisheries Aquatic Sci. FA-12.
55. Meghvansi MK, Siddiqui S, Khan MH, Gupta VK, Vairale MG, Gogoi HK, Singh L. 2010. Naga chilli: a potential source of capsaicinoids with broad-spectrum ethnopharmacological applications. J Ethnopharmacol. 132:1–14.
56. Ravishankar GA, Suresh B, Giridhar P, Rao SR, Johnson TS. Biotechnological studies on Capsicum metabolite production and plant improvement. In: De AK, editor. Capsicum: The genus Capsicum. London: CRC Press; 2003. p. 100.
57. Kothari, S.L., A. Joshi, S. Kachhwaha, N. Ochoa-Alejo. 2010. Chilli peppers – A review on tissue culture and transgenesis
58. Govindarajan VS. Capsicum — production, technology, chemistry and quality. III. Chemistry of the color, aroma, and pungency stimuli. Crit Rev Food Sci Nutr 1986;4:245–355.
59. Tripathi S, Mishra HN. 2009. Nutritional changes in powdered red pepper upon in vitro infection of Aspergillus flavus.
60. Ismail F, Anjum MR, Mamon AN, Kazi TG. 2011. Trace metal contents of vegetables and fruits of Hyderabad retail market. Pak J Nutr. 10:365–372.
61. Simonne AH, Simonne EH, Eitenmiller RR, Mills HA, Green NR. Ascorbic acid and provitamin A contents in unusually colored bell peppers (Capsicum annuum L.). J Food Compo Anal 1997;10:299–311.
62. Suzuki K, Mori M. Carotenoid composition of new cultivar of Capsicum annuum during maturation and its high capsanthin content. J Japan Soc Food Sci Tech 2003;50: 324–6.
63. Thapa, B., N. Skalko-Basnet, A. Takano, K. Masauda, and P. Basnet. 2009. High-performance liquid chromatography analysis of capsaicin content in 16 *Capsicum* fruits from Nepal. J Med Food 12(4) 908-913. DOI: 10.1089/jmf.2008.0187
64. Farhan A. Khan, T. Mahmood, M. Ali, A. Saeed, and A. Maalik. 2014. Pharmacological importance of an ethnobotanical plant: Capsicum annuum L., Natural Product Research: Formerly Natural Product Letters, 28:16, 1267-1274, DOI: 10.1080/14786419.2014.895723.
65. Prasad, B. C. N., Kumar, V., Gururaj, H. B., Parimalan, R., Giridhar, P., & Ravishankar, G. A. (2006). Characterization of capsaicin synthase and identification of its gene (csy1) for pungency factor capsaicin in pepper (Capsicum sp.). PNAS, 103, 13315–13320.
66. Escogido, M. L. R., E. G. G. Mondragon, and E. V. Tzompantzi. 2011. Review: Chemical and pharmacological aspects of capsaicin. Molecules 16, 1253-1270; doi: 10.3390/molecules16021253.

67. Giuffrida, D., P. Dugo, G. Torre, C. Bignardi, A. Cavazza, C. Corradini, G. Dugo. 2013. Characterization of 12 Capsicum varieties by evaluation of their carotenoid profile and pungency determination.
68. Zewdie, Y. and P.W. Bosland. 2000. Evaluation of genotype, environment, and genotype-by- environment interaction for capsaicinoids in Capsicum annuum L. Euphytica 111:185–190
69. Masser, M. P., J. Rakocy, and T. M. Losordo. 1999. Recirculating aquaculture tank production systems: management of recirculating systems. Southern Regional Aquaculture Center Publ. No. 452.
70. Villaverde, S., P. A. Garcia-Encina, and F. Fdz-Polanco, 1997. Influence of pH over nitrifying biofilm activity in submerged biofilters. Water Res. 31:1180-1186.
71. Ochoa-Alejo, N.; Ramírez-Malagón, R. *In vitro* chili pepper biotechnology. *In Vitro Cell. Dev. Biol. Plant.* **2001**, *37*, 701-729.
72. Estrada, B.; Pomar; F.; Díaz, J.; Merino, F.; Bernal, M.A. Pungency levels in fruits of the padron pepper with different water supply. *Hort. Sci.* **1999**, *81*, 385-396.
73. Mueller-Seitz, E.; Hiepler, C.; Petz, M. Chili pepper fruits: content and pattern of capsaicinoids in single fruits of different ages. *J. Agric. Food Chem.* **2008**, *56*, 12114-12121.
74. Varindra, P. and S. Sharma. Accumulation of Capsaicin in Seed, Peripcarp and Placenta of *Capsicum annuum* L Fruit. *J. Plant Biochemistry & Biotechnology.* 2008, 17(1), 23-27.
75. Springmann, M., C. J. Godfray, M. Rayner, P. Scarborough. Analysis and valuation of the health and climate change cobenefits of dietary change. PNAS. 2016, 113(15), 4146-4151.
76. Kehie, M., S. Kumaria, P. Tandon, N. Ramchiary. Biotechnological advances on in vitro capsaicinoid biosynthesis in *capsicum*: a review. *Phytochem Rev.* 2015, 14: 189-201.
77. Serrano M, Zapata PJ, Castillo S, Guille´n F, Mart´ınez-Romero D, Valero D. 2010. Antioxidant and nutritive constituents during sweet pepper development and ripening are enhanced by nitrophenolate treatments. Food Chem. 118:497 – 503.
78. Tokuyama, T., A. Mine, K. Kamiyama, R. Yabe, K. Satoh, H. Matsumoto, R. Takahashi, and K. Itonaga. 2004. *Nitrosomonas communis* strain YNSRA, an ammonia-oxidizing bacterium, isolated from the reed rhizoplane in an aquaponics plant. J. Biosci. Bioeng., 98 (4), 309-312.
79. Mazzone SB, Geraghty DP (1999) Respiratory action of cap- saicin microinjected into the nucleus of the solitary tract: involvement of vanilloid and tachykinins receptors. Br J Pharmacol 127:473–481
80. Ramakrishna, R., Shipton, T.A., Hasan, M.R., 2013. Feeding and feed management of Indian major carps in Andhra Pradesh, India. FAO Fisheries and Aquaculture Technical Paper No. 578. FAO, Rome (90 pp.).